Studies of Phosphodiesterase Type 4

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Contents

Chapter 1.

Preface

Atopic dermatitis is the most common skin disease, affecting a large number of children, adults, and their families (Table 1-1) (1). Worldwide prevalence of atopic dermatitis ranges from 0.2 to 25%, and the prevalence in developing countries has markedly increased in recent decades. In developed countries with the highest prevalence, the disease has reached a steady level (2, 3). The natural course of atopic dermatitis varies. In one type, initial onset is in infancy (early onset) then subsides before age 2; another type starts in infancy then gradually subsides; a type that once cured, recurs after puberty; and a type with initial onset at age 5 or over (4). When initial onset is in infancy, it is frequently accompanied by food allergies. In some patients, it is known to occur with bronchial asthma, allergic rhinitis and conjunctivitis (5).

Atopic dermatitis is defined as a disease characterized primarily by pruritic eczema that repeats a pattern of remission and exacerbation, and most frequently occurs in patients with atopic diathesis. Atopic dermatitis is diagnosed when the following three symptoms are clinically presented, regardless of the severity of symptoms: *pruritus*, a *characteristic rash and distribution*, and a *chronic or relapsing course* (6, 7, 8). The pathogenesis of atopic dermatitis is attributed to complex interactions between the environment and host susceptibility genes, which alter skin barrier function and the immune system (9). Most patients with atopic dermatitis can control their skin disease

with topical therapy and skin care (Figure 1-1). However, eczematous skin lesions in some patients do not respond to treatment with moisturizers, topical corticosteroids or topical calcineurin inhibitors, while some patients experience immediate flare-ups after tapering topical anti-inflammatory therapy (1, 10). Furthermore, sleep loss due to itching are significant, and affect not only patient's quality of life (QOL) but also QOL of the patient's caregiver such as parent (11). There is an unmet need for new medical treatment options for these patients and their caregivers.

Atopic dermatitis is a subtype of eczema/dermatitis, and infiltration of inflammatory cells including lymphocytes, eosinophils, and mast cells are present in eczema lesions (12, 13). Leukocytes from patients with atopic dermatitis display reduction of cyclic adenosine monophosphate (cAMP) responses, which is an intracellular signal molecule associated with various cellular responses (14). Intracellular cAMP levels are controlled by adenylate cyclase and phosphodiesterase (PDE) (Figure 1-2). PDE include a number of different isozymes (Table 1-2), and PDE type 4 (PDE4), the one most involved in this type of inflammatory reaction, plays an important role in activation of inflammatory monocytes and T cells (15, 16). PDE4 activity is elevated in the leukocytes of patients with atopic dermatitis, and leading to leukocyte hyperactivity and inflammation (17–19).

Although inhibition of PDE4 activity in inflammatory cells is a new target of atopic dermatitis and several PDE4 inhibitors have been developed, their use has thus far been compromised by the occurrence of mechanism-associated adverse reactions, including nausea, vomiting, headache, and weight loss, which often limit the maximum tolerated dose (20, 21). Therefore, to minimize systemic exposure, a topically active PDE4 inhibitor with low transdermal bioavailability could be clinically useful.

E6005 (molecular weight: 472.49) is developed as a novel PDE4 inhibitor for the topical treatment of atopic dermatitis (Figure 1-3). E6005 shows potent and selective inhibition of PDE4, and suppresses cytokine production by lymphocytes and monocytes (22). In hapten-elicited mice models, the topical application of E6005 produces anti-inflammatory effects, with reduced expression of cytokines and adhesion molecules. In addition, topical E6005 ameliorates the appearance of atopic dermatitis-like skin lesions in hapten- and mite-elicited models (22, 23). The use of ^{14}C-labeled E6005 shows rapid clearance from the blood and low distribution to the brain, contributing to the low emetic potential (22).

Additionally, depolarization of dorsal root ganglion neurons by capsaicin (a transient receptor potential vanilloid 1 activator) is attenuated by E6005 as well as by forskolin (a cAMP elevator). E6005 elevated intracellular levels of cAMP in dorsal root ganglion cells (24). Alternatively, in NC/Nga mice with chronic dermatitis, topical E6005 inhibits spontaneous hind-paw scratching, an itch-associated response and spontaneous activity of the cutaneous nerve. The cutaneous concentration of cAMP is significantly decreased in mice with chronic dermatitis, and this decrease is reversed by topical E6005 application (25). Topical E6005 inhibits scratching and cutaneous nerve firing induced by SLIGRL-NH2 (a proteinase-activated receptor 2 agonist peptide). PDE4 subtypes are mainly expressed in keratinocytes and mast cells in the skin (26).

These results suggest that E6005 may be a promising novel therapeutic agent with antipruritic activity for the treatment of atopic dermatitis. Therefore, in chapter 2, I plan to conduct a randomized, investigator-blind, vehicle-controlled, multiple ascending dose study to evaluate the safety and pharmacokinetics of E6005 ointment in healthy volunteers (27). I show that E6005 ointment (0.01–0.2%) does not induce skin irritation,

light urticaria, or phototoxicity, and that E6005 application for 5 days is well tolerated. No clinical concerns are found in terms of adverse events, clinical laboratory tests, vital signs, electrocardiograms, and ophthalmology. Plasma concentrations of E6005 and M11 (Figure 1-3), a hydrolyzed metabolite, are below the limit of quantification at all the sampling points. This result suggests that topical application of E6005 ointment results in very low systemic exposure to E6005 in healthy volunteers.

In chapter 3, I plan to conduct a randomized, investigator-blinded, vehicle-controlled, multiple ascending dose study to evaluate the safety, tolerability and pharmacokinetics of E6005 ointment in patients with atopic dermatitis, and to assess the efficacy of E6005 (27, 28). I show that E6005 ointment (0.01–0.2%) application for 10 days is safe and well tolerated. Although E6005 is not detected in the plasma of any patients, the metabolite M11 is detected in the plasma of three. This result suggests absorption of E6005 is restricted by the stratum corneum, absorbed E6005 is rapidly metabolized to M11, and it is eliminated from the systemic circulation in humans. Patients receiving the 0.1% or 0.2% E6005 treatment have significantly greater improvement in most efficacy parameters than patients receiving the vehicle treatment. These results suggest that E6005 ointment can provide a new treatment option for atopic dermatitis that avoids safety concerns associated with PDE4 inhibitors, while maintaining similar effectiveness.

Table 1-1 Population (000s) & Prevalence (%)

Age	Japan	US	UK	France	Germany	Spain	Italy	Total
Infant	1,180	4,455	713	797	734	493	569	8,941
(0-4)	(20.3%)	(21.5%)	(21.5%)	(21.5%)	(21.5%)	(21.5%)	(21.5%)	
Child	2,160	7,123	1,047	687	539	186	310	12,052
(5-14)	(18.0%)	(17.2%)	(14.6%)	(9.4%)	(6.8%)	(4.6%)	(5.7%)	
Adult	3,646	7,960	1,634	1,647	2,351	1,229	1,652	20,119
(≥15)	(3.3%)	(3.3%)	(3.3%)	(3.3%)	(3.3%)	(3.3%)	(3.3%)	
Total	6,986	19,538	3,394	3,131	3,624	1,908	2,531	41,112
								(5.6%)

DATAMONITOR. Stakeholder Opinion: Atopic Dermatitis (Reference Code: DMHC2279). 2007.

Table 1-2 PDE family

Family	Gene	Sub-strate	Tissue/ Cellular	Function	Inhibitor
(All)					**Theophylline** is an unspecific PDE inhibitor, a currently approved treatment for asthma or other lung diseases (eg, emphysema, bronchitis). Common side effects: headache, nausea, diarrhea, trouble sleeping
PDE1	PDE1A PDE1B PDE1C	cAMP cGMP	Brain Smooth muscle Heart etc	Calcium/calmodulin-regulated PDE PDE1A probably serves to regulate vascular smooth muscle contraction and may play a role in sperm function PDE1B is involved in dopaminergic signaling as well as immune cell activation and survival PDE1C is required for vascular smooth muscle cell proliferation and may also regulate sperm function and neuronal signaling	**Vinpocetine** is a Dietary supplement of memory enhancement and cognitive improvement
PDE2	PDE2A		Adrenal medulla, Brain Heart etc	cGMP-stimulated PDE PDE2 frequently mediates cross-talk between cGMP and cAMP pathways; it regulates aldosterone secretion from the adrenal gland, cAMP and PKA phosphorylation of Ca_2 channels in the heart, cGMP in neurons, long-term memory, and barrier function of endothelial cells under inflammatory conditions	**EHNA** (erythro-9-(2-hydroxy-3-nonyl)adenine) is investigated for improving memory in animal models.
PDE3	PDE3A PDE3B	cAMP	Heart Smooth muscle Platelets Adipocyte etc	cGMP-inhibited PDE PDE3A regulates cardiac contractility, platelet aggregation, vascular smooth muscle contraction, oocyte maturation, and regulation of renin release PDE3B mediates insulin signaling, especially its antilipolytic effects; PDE3B also regulates cell cycle/proliferation and mediates the inhibitory effects of leptin and other signals on insulin secretion and renin release	**Milrinone** is a currently approved treatment for short term congestive heart failure **Cilastazol** is a treatment for intermittent claudication Common side effects: diarrhea, headache, and nausea
PDE4	PDE4A PDE4B PDE4C PDE4D	cAMP	a variety of tissues	cAMP-specific PDE At least one form is expressed in most cells, and PDE4s play roles in a wide array of processes, including brain function, monocyte and macrophage activation, neutrophil infiltration, vascular smooth muscle proliferation, fertility, vasodilation, and cardiac contractility	**Roflumilast** is a currently approved treatment for chronic obstructive pulmonary disease **Apremilast** is a currently approved treatment for psoriasis and psoriatic arthritis Common side effects: diarrhea, headache, and nausea
PDE5	PDE5A	cGMP	Platelets Smooth muscle Brain etc	cGMP-binding, cGMP-specific PDE PDE5 is a well-documented regulator of vascular smooth muscle contraction, especially in penis and lung; it is involved in NO-cGMP signaling in platelets to control aggregation and may also play a role in regulation of cGMP signaling in the brain	**Sildenafil, vardenafil, and tadalafil** are a currently approved treatment for erectile dysfunction drugs and pulmonary hypertension. Common side effects: headache, flushing, dyspepsia, abnormal vision, nasal congestion, back pain, myalgia, nausea, dizziness, and rash

Family	Gene	Sub-strate	Tissue/ Cellular	Function	Inhibitor
PDE6	PDE6A PDE6B PDE6C	cGMP	Retina	Photoreceptor PDE6 is involved in signal transduction of the photoresponse in the eye; it may also regulate melatonin release from the pineal gland	
PDE7	PDE7A PDE7B	cAMP	Immune cell Skeletal muscle Brain etc	Rolipram-insensitive PDE PDE7 is implicated to play a role in T-cell activation and activation of other inflammatory cells	
PDE8	PDE8A PDE8B	cAMP	Immune cell Liver, Kidney Testis, Thyroid etc	cAMP-specific PDE PDE8 may play a role in T cell activation, sperm, or leydig cell function	
PDE9	PDE9A	cGMP	Brain Kidney etc	cGMP-specific PDE The function of PDE9 is currently unknown, but it has been postulated to regulate NO-cGMP signaling in the brain	
PDE10	PDE10A	cAMP cGMP	Brain Testis etc	cAMP-inhibited, dual-substrate PDE PDE10A is thought to be a regulator of cGMP in the brain and may play a role in learning and memory	
PDE11	PDE11A	cAMP cGMP	Skeletal muscle Testis Prostate etc	Dual-substrate PDE PDE11 possibly has a role in sperm development and function	

Bender AT, Beavo JA. (2006) Cyclic Nucleotide Phosphodiesterases: Molecular Regulation to Clinical Use. *Pharmacol Rev*. **58**, 488 – 520.

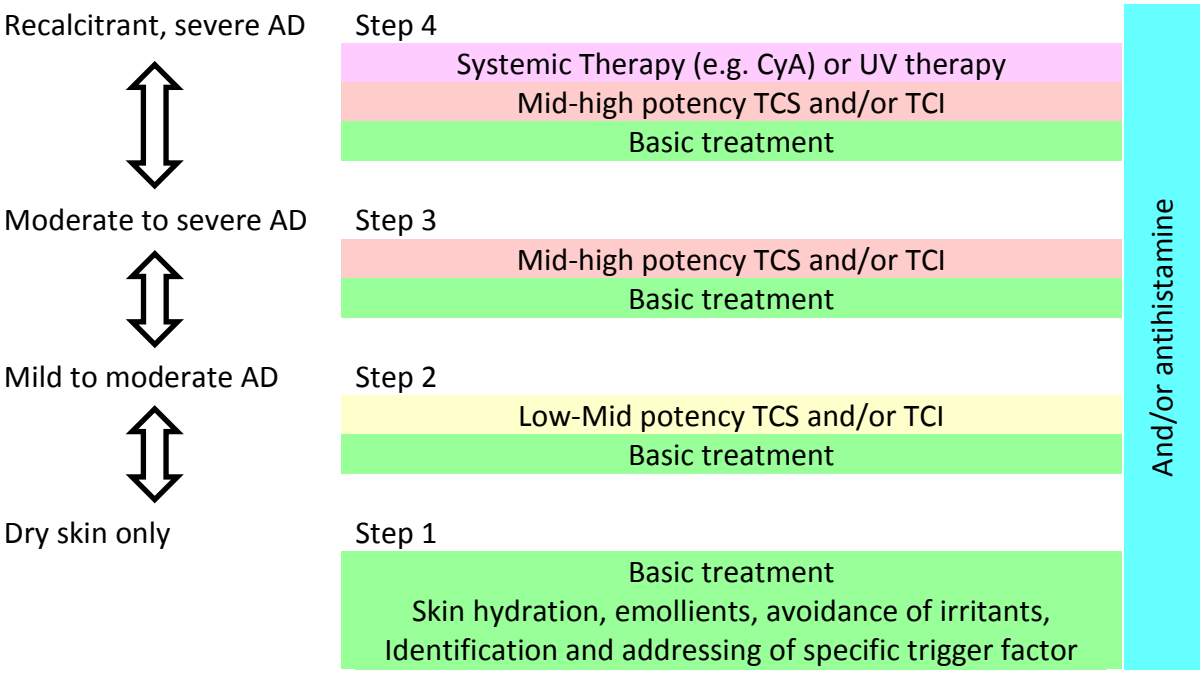
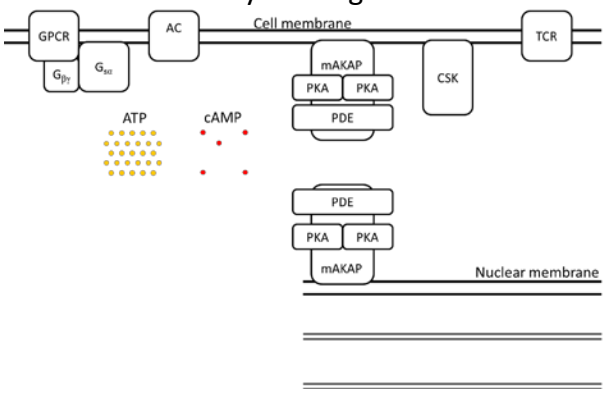


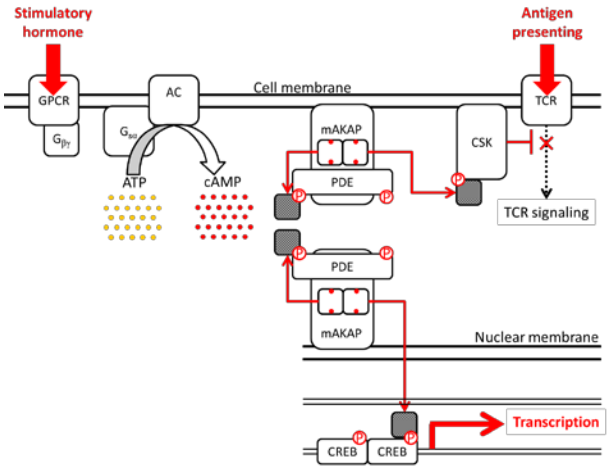
Figure 1-1 Stepwise management of patients with atopic dermatitis

AD: atopicdermatitis, CyA: cyclosporin A, TCI: topical calcineurin inhibitor, TCS: topical corticosteroid, UV: ultraviolet

A Basal PDE activity: resting state



B Increased cAMP: PKA activation



C PDE phosphorylation and activation: reduction in cAMP level

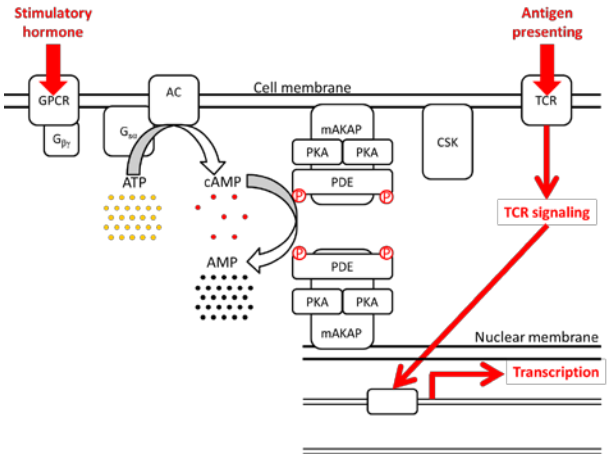
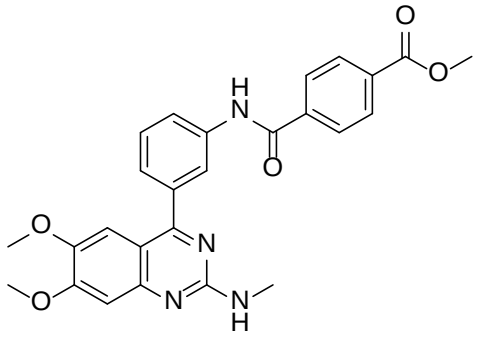


Figure 1-2 Regulation of cAMP level

AC: adenylyl cyclase, AMP: adenosine monophosphate, ATP: adenosine triphosphate, cAMP: 3',5'-cyclic AMP, CREB: cAMP response element binding protein, CSK: C-terminal Src kinase, $G_{\beta\gamma}$: G protein βγ subunit, $G_{s\alpha}$: stimulatory G protein α subunit, GPCR: G protein coupled receptor, mAKAP: membrane A-kinase anchor protein, PDE: phosphodiesterase, TCR: T-cell receptor

A E6005



B M11 (a hydrolyzed metabolite)

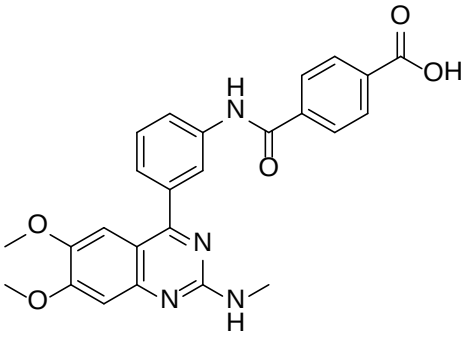


Figure 1-3 E6005 and M11

Chapter 2.

A Phase I, Multiple-dose Study of E6005 Applied Topically to the Skin of Healthy Japanese Adult Subjects

Summary

Objectives: The purpose of the present study was to assess the safety, tolerability and pharmacokinetics of topical application of a novel phosphodiesterase inhibitor, E6005, in healthy volunteers.

Methods: A randomized, investigator-blind, vehicle-controlled study was conducted to evaluate the topical application of E6005 ointment at concentrations ranging from 0.01% to 0.2% in healthy volunteers (Study 001).

Results: Thirty-six subjects were enrolled in Study 001. Neither skin irritation nor photosensitization was observed with application of E6005 in Study 001. Four subjects receiving E6005 in Study 001 experienced a treatment-emergent adverse event (application site edema, increased alanine aminotransferase or erythema); three of these subjects discontinued the study. Plasma concentrations of E6005 were below the limit of quantification (1 ng/ml).

Conclusion: E6005 ointment exhibited acceptable safety and tolerability. Topical application of E6005 ointment resulted in very low systemic exposure to E6005 in healthy volunteers.

Introduction

Atopic dermatitis is a chronic, inflammatory, pruritic skin disease that affects large numbers of children, adults and their families in industrialized countries (7). The pathogenesis of atopic dermatitis is attributed to complex interactions between the environment and host susceptibility genes, which alter skin barrier function and the immune system (29). It has been known since the 1980s that leukocytes from patients with atopic dermatitis display reduced cyclic adenosine monophosphate (cAMP) responses to stimulation (14) and elevated phosphodiesterase (PDE) activity, leading to leukocyte hyperactivity and inflammation (17–19).

Several PDE type 4 (PDE4) inhibitors have been developed to treat chronic inflammatory disorders. For example, roflumilast is approved as an oral add-on therapy for chronic obstructive pulmonary disease (30), and apremilast has been developed to treat psoriasis (31). However, the clinical utility of PDE4 inhibitors has so far been compromised by the occurrence of mechanism associated adverse reactions, including nausea, vomiting, headache and weight loss, which often limit the maximum tolerated dose (20, 21). However, a topically active PDE4 inhibitor with low transdermal bioavailability could be clinically useful.

To minimize systemic exposure, E6005 (molecular weight: 472.49) was developed as a novel PDE4 inhibitor for topical use (22). E6005 has shown potent and selective inhibition of PDE4, and suppresses the production of proinflammatory cytokines by human lymphocytes and monocytes (22). In mouse models, E6005 ointment application has shown an immediate antipruritic effect, as well as an anti-inflammatory effect, with reduced expression of cytokines and adhesion molecules

(22). A randomized, investigator-blind, vehicle-controlled, multiple ascending dose study was conducted to evaluate the safety and pharmacokinetics of E6005 ointment in healthy volunteers.

Materials and Methods

E6005 ointment in a base of white petrolatum (Eisai Co., Ltd., Tokyo, Japan) was evaluated. The primary objective of E6005-J081-001 (Study 001) was to assess the safety and pharmacokinetics of E6005 applied repeatedly to the skin of healthy Japanese men at concentrations of 0.01%, 0.03%, 0.1% or 0.2%, compared with vehicle. This study was conducted at the Sekino Clinical Pharmacology Clinic, Tokyo, Japan, in 2009. Study 001 was performed in full compliance with the International Conference on Harmonisation, all applicable Good Clinical Practice guidelines and local regulations in Japan, and was approved by the institutional investigational review boards. All the patients provided written informed consent.

Study subjects

Eligible participants met all of the inclusion criteria but none of the exclusion criteria were enrolled in the study.

Inclusion Criteria:

- Healthy Japanese adult males
- Subjects who were given a full explanation about the objective and details of this study before starting screening and who gave written consent based on their free will
- Subjects who were ≥20 years old and <45 years old at the time of obtaining written consent
- Body Mass Index (BMI [kg/m^2] = weight [kg] / height [m] × height [m]) was ≥18.5 kg/m^2 and <25.0 kg/m^2 at the time of screening

- Subjects who were considered to be eligible for study entry by the investigator or subinvestigator based on the screening assessment performed within 4 weeks prior to the first drug application

Exclusion Criteria:

- Subjects with a present illness or a history of allergy to drugs or foods, or clinically significant allergy (e.g., metal allergy, chronic asthma)
- Subjects with cutaneous hypersensitivity or photosensitivity to any dermatologic agents (e.g., plaster, transdermal drugs)
- Subjects with dermatitis or eczema, or those diagnosed by the investigator or subinvestigator as having other abnormal dermal conditions
- Subjects with gastrointestinal, hepatic, renal, respiratory, endocrine, hematological, neurological, psychiatric, or cardiovascular disorders and/or inborn error of metabolism that may influence the evaluation of the investigational drug at the time of screening or within 4 weeks prior to the first drug application
- Subjects with a known history of any surgical treatment (e.g., hepatectomy, nephrotomy, gastrointestinal resection) that may affect the pharmacokinetics of the investigational drug
- Subjects who experienced a 10% or more weight gain or loss between the time of screening and the day before the first drug application
- Subjects who consumed caffeine-containing beverage or food (coffee, tea, chocolate, coke, etc.) within 72 hours prior to the first drug application
- Subjects who consumed alcohol within 72 hours prior to the first drug application
- Subjects who smoked or consumed nicotine-containing material within 4 weeks prior to the first drug application

- Subjects who engaged in heavy exercise or labor within 2 weeks prior to the first drug application
- Subjects who were found to have clinically abnormal findings requiring medical treatment(s) in the medical history, signs and symptoms, vital signs, ECG or laboratory tests, or those with impaired organ functions
- Subjects with QTc >450 msec in the screening 12-lead ECG
- Subjects whose systolic blood pressure was ≥130 mmHg and/or diastolic blood pressure was ≥85 mmHg at the time of screening
- Subjects whose pulse rate was <50 bpm and/or ≥100 bpm at the time of screening
- Subjects with a known or suspected history of alcohol or drug abuse, or those who were positive for urine drug screening
- Subjects who received prescription drugs within 4 weeks prior to the first drug application
- Subjects who received OTC medications, nutritional supplements, vitamins, and/or herbal preparations (including oriental medicines) within 1 week prior to the first drug application. Subjects who received herb preparations that are known to be cytochrome P450 enzyme inducer (e.g., St. John's Wort preparations) within 4 weeks prior to the first drug application
- Subjects who received another investigational drug or used another investigational medical device within 16 weeks prior to the first drug application
- Subjects who received blood transfusion within 12 weeks prior to, those who donated 400 mL or more whole blood within 12 weeks prior to, those who donated 200 mL or more whole blood within 4 week prior to, or those who donated component blood within 2 weeks prior to the first drug application

- Subjects with a history of infection requiring medical treatment within 4 weeks prior to the first drug application
- Subjects who were positive for hepatitis B surface antigen (HBs antigen), hepatitis C virus (HCV) antibody, or a serologic test for syphilis
- Subjects who were diagnosed with acquired immunodeficiency syndrome (AIDS), or those who were positive for the human immunodeficiency virus (HIV) antibody
- Subjects who were unwilling to or unable to abide by the requirements of this study
- Subjects whom the investigator or subinvestigator considered ineligible for study entry

Investigational drugs

In present study, white petrolatum, E6005 (Methyl 4-[({3-[6,7-dimethoxy-2-(methyl amino)quinazolin-4-yl]phenyl}amino)carbonyl]benzoate, molecular weight: 472.49) ointment and vehicle (ointment base that did not include E6005) were used. E6005 ointment and vehicle were manufactured by Eisai Co, Ltd.

Study design

In Study 001, participants were divided into four cohorts of nine subjects each, according to the concentration of E6005 ointment applied (0.01%, 0.03%, 0.1% or 0.2%). Seven subjects in each cohort were randomly selected to receive E6005 ointment application, while two received vehicle application. Each cohort participated in two testing periods (Figure 2-1, Table 2-1). In Period I, approximately 30 mg of the investigational drugs (no treatment; white petrolatum; vehicle; 0.01%, 0.03%, 0.1% and 0.2% E6005) were individually placed in two sets of Finn Chambers® (Epitest Ltd Oy,

Tuusula, Finland) for patch and photopatch testing. The Finn Chambers® were applied to the outer surface of the upper arms on day 1. In the first half of Period II, a simple application of the investigational drug was applied to the posterior trunk at dosages of 1 g in the morning on day 9, 2 g in the morning on day 11 and 5 g in the morning on day 13. In the second half of Period II, on days 15–19, repeated 5 g doses were applied in the morning and at night. The type of ointment applied (E6005 or vehicle) was masked to assessment investigators and to subjects. When no safety concerns arose within a cohort, the next cohort was started with a higher E6005 concentration.

Assessments

In the patch test (skin irritation test), the investigational drugs were applied to the skin for 48 h. Skin reactions were assessed 30 min and 24, 72 and 120 h after removing the Finn Chamber®, according to the criteria of the International Contact Dermatitis Research Group (32) and Japanese criteria (33).

In the photopatch test (photosensitivity test), the investigational drugs were applied for 24 h. The skin was then exposed to long-wavelength irradiation (UVA, 6 J/cm^2). The presence or absence of light urticaria was assessed 30 min after optical irradiation, and the site was then covered with a new empty Finn Chamber® for 1 day. Skin reactions were assessed 24, 48, 96 and 144 h after optical irradiation, according to the criteria of the International Contact Dermatitis Research Group (34) and Japanese criteria (33).

All the skin reactions were assessed by a physician who was kept blind to the preparation sequence. The assessor did not apply or remove the preparations during the study and was never told the preparation sequence. A photograph of the application site

was taken before application and at the time of assessment.

The safety profile was assessed with treatment-emergent adverse event reporting, based on the Common Terminology Criteria for Adverse Events (National Cancer Institute, Bethesda, MD, version 4.02), clinical laboratory testing, electrocardiogram recording, vital signs and ophthalmological findings.

Measurement of plasma concentrations of E6005 and its metabolite

In Study 001, blood samples were collected for assessment of pharmacokinetics before initial treatment, before dosing on days 8, 9, 11, 13, 15 and 19, and at 1-, 2-, 4- and 8-h post-dose on days 15 and 19. Plasma concentrations of E6005 and its major metabolite (the methyl ester hydrolysis product) were quantified with a validated liquid chromatography-tandem mass spectrometry method with a lower limit of quantification of <1 ng/ml (35).

Statistical analysis

Randomization schedules were generated by the SAS RANUNI procedure. All the analyses were performed by Eisai Co., Ltd. using SAS® for Windows (SAS Institute, Cary, NC, version 8.2 or later).

The full analysis set population was used for all the analyses. Data were summarized descriptively according to treatment. For continuous variables, descriptive statistics are generally presented where applicable (including number of subjects [N], percentage of subjects [%], arithmetic mean [mean] and standard deviation [SD]).

Results

Demographics and baseline characteristics

In Study 001, 36 healthy male subjects were randomized: 28 to active treatment and 8 to vehicle. Thirty-three of these completed the study and three did not (two because of adverse events and one because of subject choice) (Figure 2-1). Demographics and baseline characteristics were generally similar among all the treatment groups (Table 2-2).

Skin irritation and photosensitivity

In the skin irritation test in Study 001, four subjects had a positive reaction 30 min after removal of the Finn Chamber® (Table 2-3). One subject, who experienced application site edema without erythema at the site of the ‘‘no application’’ chamber as a treatment-emergent adverse event, improved without treatment and the reaction was considered not related to the study drug. Three subjects experienced faint application site erythema, one at the site of no treatment, one at the white petrolatum site and one at the 0.2% E6005 site. These were not classified as adverse events, because assessment investigators considered the erythema in these cases to be related to physical contact with the chamber. Photosensitivity testing was negative in all the participants.

Safety and tolerability

In Study 001, 4 of 28 subjects (14.3%) in the E6005 ointment treatment group and 1 of 8 (12.5%) in the vehicle treatment group had at least one treatment-emergent adverse event (Table 2-4). One subject, who experienced application site edema in the

patch test/ photopatch test, is described earlier. Two subjects in the 0.1% E6005 ointment group experienced increased alanine aminotransferase. One discontinued the study on day 13 because of subject choice and improved without treatment. This elevation was considered to be possibly related to the study drug. The second participant continued the study, improved without treatment and the elevation was considered not related to the study drug. One subject in the 0.2% E6005 ointment treatment group and one in the vehicle treatment group in Cohort 3 experienced erythema, which occurred in the application area and in a non-application area. Both participants discontinued the study on Day 16, improved without treatment and the reactions were considered not related to the study drug. Deaths and other serious or severe adverse events (Grade 3 or higher) were not observed. Laboratory parameters, vital signs, electrocardiogram parameters and ophthalmological examination findings did not change clinically.

Pharmacokinetic profile

Plasma concentrations of E6005 and the metabolite M11 were below the lower limit of quantification (1 ng/ml) at all the sampling points in all the subjects, so it was not possible to obtain any meaningful pharmacokinetic data (Table 2-5).

Discussion

Neither skin irritation nor photosensitization was observed in subjects treated with E6005 ointment or vehicle ointment, according to Japanese and International Contact Dermatitis Research Group criteria. In addition, there was no specific adverse reaction reported at any E6005 concentration up to 0.2% in this study. These results, including the assessments according to Japanese criteria (33), indicate no safety concerns for skin application of E6005 ointment or vehicle, including skin irritation, phototoxicity or light urticaria.

Burning sensation and pruritus at the site of application, which are the commonly reported adverse reactions associated with tacrolimus ointment (36), were not observed in the present study. Nausea, vomiting, headache and weight loss, which are the commonly reported adverse reactions associated with other PDE4 inhibitors (20, 21), were not observed in the present study.

In all the healthy volunteers, plasma concentrations of E6005 and its metabolite M11 were below the limit of quantification at all the sampling points during the 5-day repeated application of E6005 ointment. The present findings are consistent with the results obtained when testing healthy skin in rats (22).

In conclusion, the present data indicate that E6005 ointment is safe and well tolerated in healthy volunteers, although the results should be interpreted with caution because of the small number of healthy subjects included. Further studies in patients with atopic dermatitis are necessary to evaluate the safety of E6005 ointment applied to larger areas throughout the body for longer periods.

Acknowledgement

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Table 2-1 Schedule of Each Step in E6005-J081-001

	Outpatient	Inpatient							Discharge	(Outpatient)
Within-Period	Screening	Period I				Period II			(Follow-up)	(Follow-up)
Day	-28 to -1	0	1 to 3	4, 6	8	1, 3, 5	7 to 11	12		
Total Day	-28 to -1	0	1 to 3	4, 6	8	9, 11, 13	15 to 19	20	22	
Matters to be performed	Screening examinations	Admission in the study institution and prior examinations	Patch est/photopatch test	Assessment	Assessment and prior examinations for Period II	Drug application (once daily)	Drug application (twice daily)	Assessment	Follow-up examinations	(Follow-up examinations)

Table 2-2. Subject demographics and baseline characteristics

Parameter	Vehicle	E6005 ointment					All subjects
		0.01%	0.03%	0.1%	0.2%	Total	
	(n=8)	(n=7)	(n=7)	(n=7)	(n=7)	(n=28)	(n=36)
Age (years)	25.8±4.5	25.4±2.4	24.7±3.7	27.1±3.7	28.7±5.6	26.5±4.1	26.3±4.2
	22, 36	22, 29	21, 30	23, 33	22, 36	21, 36	21, 36
Height (cm)	175.34±4.26	172.93±4.42	174.59±2.90	177.36±5.98	173.13±4.11	174.50±4.60	174.69±4.48
	169.5, 181.1	168.5, 182.1	168.9, 177.2	170.4, 186.7	168.9, 180.9	168.5, 186.7	168.5, 186.7
Body weight (kg)	68.83±6.16	63.99±3.13	65.63±4.61	70.50±6.13	64.06±2.47	66.04±4.89	66.66±5.24
	62.8, 80.2	60.4, 70.1	60.6, 72.8	61.0, 78.2	60.7, 67.6	60.4, 78.2	60.4, 80.2
BMI (kg/m^2)	22.38±1.57	21.40±0.70	21.53±1.26	22.41±1.82	21.40±0.65	21.69±1.22	21.84±1.31
	21.0, 24.8	20.8, 22.4	20.3, 23.6	20.5, 24.8	20.4, 22.2	20.3, 24.8	20.3, 24.8
TEWL[a] (g/m^2h)	12.23±5.43	8.89±1.56	10.31±1.91	13.16±9.70	9.56±1.70	10.48±5.07	10.87±5.12
	7.3, 24.3	5.6, 10.1	8.1, 13.5	6.6, 34.6	6.0, 11.1	5.6, 34.6	5.6, 34.6

BMI: body mass index, SD: standard deviation, TEWL: transepidermal water loss.
Upper row: Mean±standard deviation, lower row: minimum, maximum
a: Calculated from the average of measured values on the left upper back and the right upper back

Table 2-3. Skin irritation test results (International Contact Dermatitis Research Group criteria)

Interpretation	No treatment (n=36)	White petrolatum (n=36)	Vehicle (n=36)	E6005 0.01% (n=36)	E6005 0.03% (n=36)	E6005 0.1% (n=36)	E6005 0.2% (n=36)
Skin irritation test (patch test)							
No reaction	34 (94.4%)	35 (97.2%)	36 (100%)	36 (100%)	36 (100%)	36 (100%)	35 (97.2%)
Doubtful reaction (faint erythema only)	1 (2.8%)	1 (2.8%)	0	0	0	0	1 (2.8%)
Weak positive reaction (erythema, infiltration, possibly papules)	0	0	0	0	0	0	0
Strong positive reaction (erythema, infiltration, papules, vesicles)	0	0	0	0	0	0	0
Extreme positive reaction (intense erythema, infiltration, coalescing vesicles)	0	0	0	0	0	0	0
Irritant reaction of a different type	1 (2.8%)	0	0	0	0	0	0
Photosensitivity test (photopatch test)							
Negative reaction	36 (100%)	36 (100%)	36 (100%)	36 (100%)	36 (100%)	36 (100%)	36 (100%)
Doubtful reaction; faint erythema only	0	0	0	0	0	0	0
Weak positive reaction; erythema, infiltration, passively papules	0	0	0	0	0	0	0
Strong positive reaction; erythema, infiltration, papules, vesicles	0	0	0	0	0	0	0
Extreme positive reaction; intense erythema and infiltration and coalescing vesicles	0	0	0	0	0	0	0
Irritant reaction of different type	0	0	0	0	0	0	0
Light urticaria	1 (2.8%)	0	1 (2.8%)	0	0	0	0

Table 2-4. Treatment-emergent adverse events

MedDRA System organ class and preferred term [a]	Vehicle	E6005			
		0.01%	0.03%	0.1%	0.2%
	(n=8)	(n=7)	(n=7)	(n=7)	(n=7)
Any adverse event	1 (12.5%)	1 (14.3%)	0	2 (28.6%)	1 (14.3%)
General disorders and administration site conditions					
Application site edema	0	1 (14.3%)	0	0	0
Investigations					
Alanine aminotransferase increased	0	0	0	2 (28.6%)	0
Skin and subcutaneous tissue disorders					
Erythema	1 (12.5%)	0	0	0	1 (14.3%)

a: Medical Dictionary for Regulatory Activities, version 17.1

Table 2-5. Number of subjects with plasma concentrations of E6005 or M11 above the lower limit of quantification

	Vehicle	E6005			
		0.01%	0.03%	0.1%	0.2%
	(n=8)	(n=7)	(n=7)	(n=7)	(n=7)
E6005	0	0	0	0	0
M11	0	0	0	0	0

The lower limit of quantification was 1.00 ng/mL.

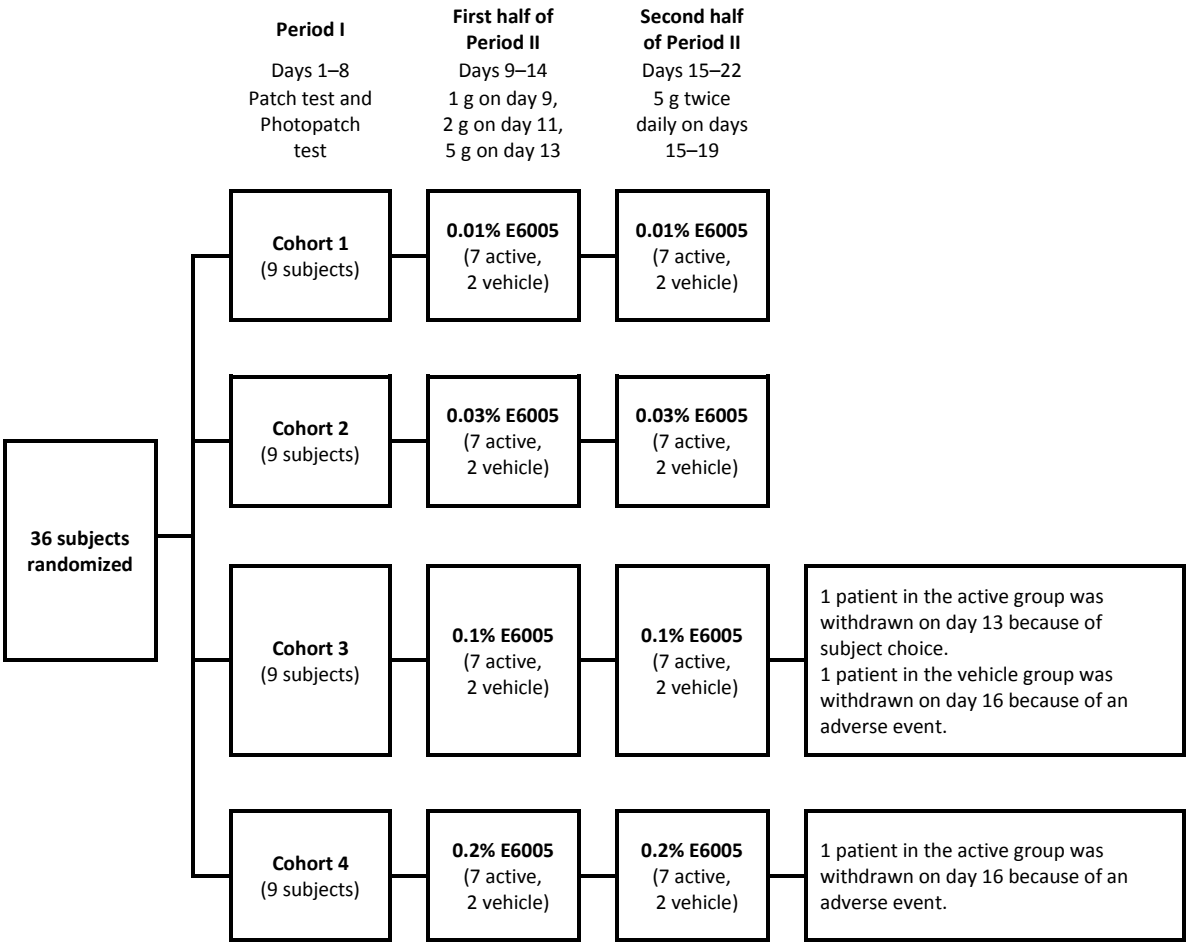

Figure 2-1. Design and subject allocation schema for Study 001.

Chapter 3.

A Phase I/II Study of E6005 in Japanese Patients with Atopic Dermatitis

Summary

Introduction: Phosphodiesterase type 4 (PDE4) inhibition is a well-known anti-inflammatory mechanism. However, the clinical use of PDE4 inhibitors has been compromised by the occurrence of mechanism-associated adverse reactions, which often limit the maximum tolerated dose. To minimize systemic exposure, a topically active PDE4 inhibitor with low transdermal bioavailability could be clinically useful. The purpose of this study was to evaluate the safety, tolerability and pharmacokinetics of topical application of a novel topical PDE4 inhibitor, E6005, and to assess the efficacy of E6005 in patients with atopic dermatitis.

Methods: This randomized, investigator-blinded, vehicle-controlled, multiple ascending dose study included 40 adult male patients with atopic dermatitis, who were randomly assigned to 10 days of treatment with either E6005 ointment (0.01, 0.03, 0.1 or 0.2%) or vehicle ointment.

Results: Of 81 patients screened, 40 who had typical lesions on their posterior trunk were randomized into the study. One patient receiving 0.03% E6005 treatment discontinued because of acute gout and one receiving vehicle treatment discontinued because of progression of atopic dermatitis. Plasma concentrations of E6005 were

below the limit of quantification (1 ng/ml). The targeted lesion severity scores decreased in a concentration-dependent manner in patients treated with E6005. This drop was significant in the 0.2% E6005 ointment treatment group (mean percent change: -54.30%, p=0.007).

Conclusion: E6005 ointment exhibited acceptable safety and tolerability. Topical application of E6005 ointment resulted in very low systemic exposure to E6005 in patients with atopic dermatitis. E6005 ointment showed anti-inflammatory efficacy in adult patients with atopic dermatitis.

Introduction

Atopic dermatitis is the most common skin disease, affecting a large number of children, adults, and their families (1). Although a high percentage of atopic dermatitis cases arise and go into remission during childhood, atopic dermatitis may also develop in adolescents and adults (9). Worldwide prevalence of atopic dermatitis ranges from 0.2 to 25%, and the prevalence in developing countries has markedly increased in recent decades. In developed countries with the highest prevalence, the disease has reached a steady level (2, 3). Most patients with atopic dermatitis can control their skin disease with topical therapy and skin care. However, eczematous skin lesions in some patients do not respond to treatment with moisturizers, topical corticosteroids or topical calcineurin inhibitors, while some patients experience immediate flare-ups after tapering topical anti-inflammatory therapy (1, 10). There is an unmet need for new medical treatment options for these patients.

E6005 (molecular weight: 472.49) was developed as a novel PDE4 inhibitor for topical use (22). PDE4 is expressed in several types of inflammatory cells (37). PDE4 activity is elevated in the leukocytes of patients with atopic dermatitis (17), and it reduces cyclic adenosine monophosphate (cAMP) responses to stimulation (14), leading to leukocyte hyperactivity and inflammation (18, 19). E6005 shows potent and selective inhibition of PDE4, and suppresses cytokine production by lymphocytes and monocytes. In mouse models, the topical application of E6005 produces anti-inflammatory effects, with reduced expression of cytokines and adhesion molecules (22). Topical E6005 treatment also showed acute antipruritic activity in a mouse model of chronic dermatitis (25), with inhibition of itch-associated protease-activated receptor 2 (26), and decreased

excitation of itch-relevant dorsal root ganglion neurons (24).

Although several PDE4 inhibitors have been developed, their use has thus far been compromised by the occurrence of mechanism-associated adverse reactions, including nausea, vomiting, headache and weight loss, which often limit the maximum tolerated dose (20, 21). To minimize systemic exposure, a topically active PDE4 inhibitor with low transdermal bioavailability could be clinically useful. It previously reported that the topical application of E6005 ointment was safe and well tolerated in healthy volunteers (27). The objective of the present study was to evaluate the safety, tolerability and pharmacokinetics, and to assess the efficacy of topical E6005 ointment in male Japanese patients with atopic dermatitis. The outcome assessments in this trial were the severity scores of targeted lesions on the back, the Severity Scoring of Atopic Dermatitis (SCORAD) indexes (38), the Eczema Area and Severity Index (EASI) scores (39) and laboratory parameters.

Materials and Methods

This randomized, investigator-blinded, vehicle-controlled study to assess the safety and efficacy of E6005 ointment was undertaken at the Kyushu Clinical Pharmacology Research Clinic in Japan. The study was performed in full compliance with the International Conference on Harmonization, all applicable Good Clinical Practice guidelines, and local regulations in Japan. The study was approved by the institutional investigational review board and registered at www.ClinicalTrials.gov (NCT01179880). All patients provided written informed consent.

Study population

Patients eligible for the present study were Japanese men aged 20 to 65 years with mild-to-severe atopic dermatitis, diagnosed according to the ‘‘Guidelines for Management of Atopic Dermatitis’’ of the Japanese Dermatological Association (40). To be included, patients needed to have evaluable typically eczematous skin lesions on their posterior trunk (targeted lesion) to explore the efficacy of topical E6005 ointment application. Patients were excluded if they had additional skin diseases that would interfere with the study results. Patients were required to stop all topical atopic dermatitis therapy except for white petrolatum (such as topical corticosteroids and calcineurin inhibitors) at least 1 week before baseline evaluation, and to discontinue any systemic therapy (antihistamines, systemic corticosteroids, cyclosporine, tacrolimus), phototherapy and photochemotherapy at least 2 weeks before baseline evaluation.

Inclusion Criteria:

- Japanese adult male patients with a confirmed diagnosis of atopic dermatitis

according to “Guidelines for Management of Atopic Dermatitis” by the Japanese Dermatological Association

- Patients with evaluable typical eczema on the back (posterior trunk)
- Male subjects aged 20 years and over and under 65 years at the time of providing written informed consent
- Virile men who and whose partner agreed to take adequate contraceptive measures during the study
- Provided written informed consent of their free will
- Were willing and able to comply with all the requirements of the protocol

Exclusion Criteria:

- Patients complicated with eye symptoms (e.g., cataract, retinal detachment), Kaposi varicelliform eruption, molluscum contagiosum or impetigo contagiosa
- Patients who had an existing condition or a history of a severe allergy such as anaphylactic shock, anaphylactic reaction and anaphylactoid reaction or drug allergy/hypersensitivity to E6005 or any of the excipients
- Patients who received any concomitant ethical drugs or any phototherapies within 14 days before Baseline. Subjects were allowed to use external steroids and tacrolimus ointment up to 8 days before Baseline and white petrolatum throughout the study period
- Patients with any infection that required hospitalization or intravenous/oral treatment with antibiotic/antiviral/antifungal drug(s) within 28 days before Baseline
- Patients who had an existing condition or a history of any malignant tumor, lymphoma, leukemia, or lymphoproliferative disorders, which does not include non-melanoma skin cancers (e.g., squamous cell carcinoma and basal cell

carcinoma) that were completely removed and have not metastasized for 5 years

- Patients who could not discontinue prohibited concomitant medication or therapy
  - From Baseline to the end of follow-up period or early discontinuation
    - Topical agents (e.g., cosmetics, pharmaceutical cosmetics). However, white petrolatum was permitted throughout the study.
    - Caffeinated food or beverage (e.g., coffee, tea, chocolate, and coke). For laboratory tests at Baseline, any caffeinated food or beverage (e.g., coffee, tea, chocolate, and coke) except for tea (e.g., black tea and green tea) was prohibited from 10 hours before Baseline.
  - From 72 hours before Baseline to the end of follow-up period or early discontinuation
    - Alcohol
  - From 7 days before Baseline to the end of follow-up period or early discontinuation
    - Topical steroids
    - Tacrolimus ointment
    - Food or beverage containing grapefruit
  - From 14 days before Baseline to the end of follow-up period or early discontinuation
    - Ethical drugs, except for topical steroids, and tacrolimus ointment
    - Phototherapies
    - Over-the-counter medications, nutritional supplements, vitamins, and herbal preparations (including Chinese medicines)
    - Blood component collection

- From 28 days before Baseline to the end of follow-up period or early discontinuation
    - Smoking or consumption of nicotine products (e.g., nicotine gum and nicotine patch)
    - Herbal preparations containing St. John's Wort known to induce cytochrome P450
    - Whole blood collection (≥200 mL, <400 mL)
- From 84 days before Baseline to the end of follow-up period or early discontinuation
    - Blood transfusion
    - Whole blood collection (≥400 mL)
- From 112 days before Baseline to the end of follow-up period or early discontinuation
    - Participation in another clinical trial of an investigational drug or device

- Patients with active syphilis, human immunodeficiency virus (HIV), or viral hepatitis (B or C) as demonstrated by positive on laboratory examination
- Patients who had an existing condition or a history of unstable ischemic heart disease, cardiac failure congestive (New York Heart Association class III or IV), cerebral infarction, or cerebral hemorrhage
- Prolongation of QTc interval (> 450 msec) demonstrated on repeated electrocardiograms (ECGs), using Bazett's or Friedericia's correction
- Suffering from psychotic disorder(s) and/or unstable recurrent affective disorder(s) evident by use of antipsychotics or had had a suicide attempt(s) within 2 years before Baseline

- History of drug or alcohol dependency or abuse within 2 years before Baseline, or current user of psychotropic drugs for recreation other than therapeutic purpose
- Presence of a progressive central nervous system (CNS) disease, including degenerative CNS diseases and progressive tumors
- Scheduled for surgery during the projected course of the study
- Evidence of any disease listed below that could affect the subject's safety or study conduct or any disease (e.g., cardiac, respiratory, gastrointestinal, renal disease) judged by the investigator(s) to be clinically significant
  - Uncontrolled diabetes mellitus (e.g., requiring insulin)
  - Active inflammatory bowel disease, or peptic ulcer
  - Autoimmune diseases (e.g., collagen disorder)
  - Inborn error of metabolism
- Indicating at least one of the following abnormal findings at the screening period:
  - Systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg
  - Heart rate <40/min, or ≥100/min
  - Body mass index (BMI) <18.0 kg/m^2, or ≥30.0 kg/m^2

    BMI (kg/m^2) = weight (kg) / [height (m) × height (m)]
  - Hemoglobin <10.0 g/dL
  - White blood cells (WBC) <3,000/μ L
  - Platelet <75,000/μ L
  - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3× upper limit of normal (ULN)
  - Total bilirubin >1.5×ULN
  - Serum creatinine >1.5×ULN

- Patients who had any condition that would make him/her, in the opinion of the investigator, unsuitable for the study

Investigational drugs

E6005 (Methyl 4-[({3-[6,7-dimethoxy-2-(methyl amino)quinazolin-4-yl] phenyl}amino)carbonyl]benzoate, molecular weight: 472.49) ointment and vehicle (ointment base that did not include E6005) were manufactured by Eisai Co, Ltd and were used in present study.

Study design

Patients were randomized into four cohorts of 10 each, according to E6005 ointment concentration (0.01, 0.03, 0.1 and 0.2%). Ointment was applied once on day 1, twice daily on days 2 through 9, and once on day 10. Eight patients in each cohort were treated with 5 g of E6005 ointment and two patients were treated with 5 g of vehicle ointment, as randomly determined using the SAS RANUNI procedure (Figure 3-1). The ointment was applied first to the targeted lesion on the back, then to other regions of the trunk, and finally to the lower limbs if sufficient ointment remained, avoiding the head, neck and upper limbs. Assessment investigators and patients were blinded to the study drug applied. When no safety concerns arose within one cohort, the next cohort began treatment at a higher drug concentration.

Assessments

The safety profile was assessed with treatment-emergent adverse event reporting, based on the Common Terminology Criteria for Adverse Events (National

Cancer Institute, Bethesda, MD, version 4.02), clinical laboratory testing, electrocardiogram recording, vital signs and ophthalmological findings.

The severity score (range: 0–15) of the targeted lesion on the back was used as an efficacy variable. The severity score was determined as the sum of intensity scores (0: none, 1: mild, 2: moderate and 3: severe) for each of five symptoms (erythema, edema and/or papulation, oozing and/or crust, excoriation, lichenification), according to the SCORAD index assessment procedure (38). Other efficacy variables included the EASI score (range: 0–72) (Figure 3-2) (39), the SCORAD index (range: 0–103) (Figure 3-3), Pruritus score (Pruritus state during the day and Pruritus state during the night, range: 0–4) (Table 3-1) (41, 42), transepidermal water loss of the targeted lesion measured with a VapoMeter (Delfin Technologies Ltd., Kuopio, Finland), total immunoglobulin E, CCL17 (thymus and activation-regulated chemokine [TARC]), eosinophil counts and lactate dehydrogenase.

Measurement of plasma concentrations of E6005 and its metabolite

In Study 101, blood samples were collected pre-dose on days 1, 2, 5, 10, 11, 12 and 13, and 1-, 2-, 4- and 8-h post-dose on days 1, 5 and 10. Plasma concentrations of E6005 and its major metabolite (the methyl ester hydrolysis product) were quantified with a validated liquid chromatography-tandem mass spectrometry method with a lower limit of quantification of <1 ng/ml (35).

Statistical analysis

The full analysis set population was used for all analyses. All the analyses were performed by Eisai Co., Ltd. using SAS® for Windows (SAS Institute, Cary, NC,

version 8.2 or later). Data were summarized descriptively according to treatment. For continuous variables, descriptive statistics are generally presented where applicable (including number of subjects [N], percentage of subjects [%], arithmetic mean [mean] and standard deviation [SD]).

Descriptive summary statistics of target lesion severity score, EASI score, SCORAD index, and laboratory test results at each assessment and changes from baseline were evaluated according to treatment group. *p* values were also determined using a paired *t*-test for exploratory purposes. Differences in the severity scores of the targeted lesion (last observation carried forward: LOCF) were evaluated with an analysis of covariance (ANCOVA), with treatment as a factor and baseline score as a covariate. Differences in EASI score and SCORAD index (observed case) were analyzed using a linear mixed-effect model, with treatment and time as factors, baseline scores as covariates, subjects as random effects, and changes or percent changes from baseline on days 5 and 11 as repeated measurements ($p<0.05$ was considered significant).

Results

Demographics and baseline characteristics

Of the 81 patients screened, 40 who had typical lesions on their posterior trunk were randomized into the study. Of the 40 patients, one patient receiving the 0.03% E6005 ointment treatment discontinued because of acute gout and one other receiving the vehicle treatment in Cohort 2 discontinued because of progression of atopic dermatitis and Kaposi's varicelliform eruption (Figure 3-4). Demographics and baseline characteristics were similar among all treatment groups (Table 3-2, 3).

Safety and tolerability

Two of 32 patients (6.3%) in the E6005 ointment treatment group and 1 of 8 (12.5%) in the vehicle treatment group had at least one treatment-emergent adverse event (Table 3-4). One patient in the 0.03% E6005 ointment treatment group had gout, which occurred in a non-drug application area and improved even after application of the drug. A patient in the 0.2% E6005 ointment treatment group had enterocolitis, which improved despite continuation of the study drug; this patient also had a history of lower abdominal pain during constipation. Another patient in the vehicle treatment group in Cohort 2 experienced aggravated atopic dermatitis and Kaposi's varicelliform eruption. None of these adverse events was considered to be related to the study drug. Deaths and other serious or severe adverse events (Grade 3 or higher) were not observed. Laboratory parameters, vital signs, electrocardiogram parameters and ophthalmological examinations did not change clinically.

Pharmacokinetic profile

Plasma concentrations of E6005 were below the lower limit of quantification (1 ng/ml) at all the sampling points in all the subjects, so it was not possible to obtain any meaningful pharmacokinetic data (Table 3-5). The metabolite M11 was detected in the plasma at levels just above the lower limit of quantification (1 ng/ml) and <3 ng/ml in 3 of 36 patients (8.3%; in two receiving 0.1% E6005 ointment treatment and in one receiving 0.2% E6005 ointment treatment).

Efficacy

The severity scores of the targeted lesions

Figure 3-5 shows representative photographs of improvement in the targeted lesion of a 20-year-old man, who received 0.2% E6005 ointment application. Results of outcome assessments are summarized in Table 3-6. The mean severity scores of the targeted lesions (LOCF) fell from 6.3/15 to 3.9/15 in the 0.03% E6005 treatment group (p<0.001, paired t-test), and from 7.0/15 to 3.3/15 in the 0.2% E6005 treatment group (p<0.001, paired t-test). The mean changes from baseline were 2.4 in the 0.03% E6005 group (p=0.046, ANCOVA) and 3.8 in the 0.2% E6005 group (p=0.001, ANCOVA), compared with 0.6 in the vehicle treatment group (Figure 3-6). The mean percent change from baseline decreased in an E6005-concentration-dependent manner. The decrease was significant in the 0.2% E6005 group at 54.30%, compared with 11.39% in the vehicle treatment group (p=0.007; ANCOVA).

EASI score

The study drug was applied to all lesions on the patient's trunk in the present

study. The mean trunk portion of the EASI score fell from 6.08/21.6 to 2.48/21.6 in the 0.1% E6005 treatment group (p=0.008, paired t-test), and from 5.66/21.6 to 3.30/21.6 in the 0.2% E6005 treatment group (p=0.002, paired t-test). Although the study drug was not applied to the head, neck or upper limbs, the mean change in EASI score total from baseline was 7.14/72 in the 0.1% E6005 group (p=0.004, analysis of linear mixed-effect model), and 6.13/72 in the 0.2% E6005 group (p=0.035, analysis of linear mixed-effect model), compared with 2.31/72 in the vehicle treatment group (Figure 3-7).

SCORAD index

Objective SCORAD is calculated based on SCORAD-A (extent of eczema) and SCORAD-B (intensity of eczema), and then SCORAD index total is calculated based on Objective SCORAD and SCORAD-C (subjective symptoms), using the following formulae (38).

Objective SCORAD=SCORAD-A / 5 + SCORAD-B × 7 / 2

SCORAD=Objective SCORAD + SCORAD-C

The mean change in the Objective SCORAD from baseline on Day 11 was 8.99/83 in the 0.1% E6005 group (p=0.016, analysis of a linear mixed-effect model), compared with 2.57/83 in the vehicle treatment group (Table 3-6). On the other hand, the SCORAD-C from baseline on Day 11 significantly reduced in the 0.03% E6005 group (p=0.038, paired t-test) and 0.1% E6005 group (p=0.003, paired t-test), but there was no statistically significant reduction compared with the vehicle group (Table 3-6). Consequently, the mean change in the SCORAD index total from baseline was 11.40/103 in the 0.1% E6005 group (p=0.015, analysis of a linear mixed-effect model), compared with 4.20/103 in the vehicle treatment group (Figure 3-8).

Pruritus score

Pruritus score is summarized in Table 3-6. Pruritus day scores were significantly reduced from baseline in the 0.01% E6005 group and 0.03% E6005 group (p=0.049 in each treatment group, paired t-test) at the end of study. For pruritus night score, there was no statistically significant reduction from baseline at the end of study in any treatment group at the end of study.

Laboratory tests

Although there were statistically significant reductions from baseline for transepidermal water loss, eosinophil counts, and lactate dehydrogenase in the E6005 treatment group at the end of the study, there was no significant reduction in total immunoglobulin E or CCL17 (Table 3-6).

Discussion

This study is to evaluate the safety, tolerability and pharmacokinetics of topical application of E6005 ointment, and the first study to assess the therapeutic effect of E6005 ointment compared with a vehicle in Japanese male patients with atopic dermatitis. Most participants had mild or moderate atopic dermatitis. E6005 ointment (0.01%, 0.03%, 0.1%, and 0.2%) was safe and well-tolerated when applied repeatedly for 10 days to patients with atopic dermatitis.

Tacrolimus ointment is widely used in treating atopic dermatitis. The most common adverse effects of tacrolimus ointment are a burning sensation and pruritus at the site of application, occurring during the first few days of treatment (36). In previous study of patients with atopic dermatitis, no application site reaction, including burning sensation and pruritus, occurred with use of E6005 ointment. Although the present studies did not compare topical E6005 with tacrolimus, the present results indicate that E6005 ointment may have a safer profile.

In all healthy volunteers, plasma concentrations of E6005 and its metabolite M11 were below the limit of quantification at all sampling points during the 5-day repeated application of E6005 ointment (27). The present findings are consistent with the results obtained when testing healthy skin in rats (22). Although E6005 was not detected in the plasma of any patient with atopic dermatitis in the present study, the metabolite M11 was detected in the plasma of three. These results suggest that the stratum corneum is a major barrier to E6005 absorption through the skin in humans (as in rats) and that a small quantity of E6005 is absorbed through eczematous lesions in humans. Furthermore, an in vitro metabolism study using liver microsomes from

humans and other animals showed rapid metabolism of E6005 to M11. Together these results suggest low absorption and rapid elimination of E6005 from the systemic circulation in humans.

Nausea, vomiting, headache, and weight loss, which are the commonly reported adverse reactions associated with other PDE4 inhibitors (20, 21), were not observed in the present studies. PDE4 inhibitors are thought to produce a pharmacological response analogous to that of presynaptic α2-adrenoceptor inhibitors by elevating intracellular levels of cAMP in noradrenergic neurons (43). One way to evaluate the emetic potential of this class of drugs is to assess the anesthesia-reversing effect of PDE4 inhibitors in rats, which do not have a vomiting reflex (44). In a study using this model of emesis, the reversal of anesthesia with systemic E6005 administration was less potent than that with cilomilast administration (22). Additionally, as discussed above, absorption of E6005 is restricted by the stratum corneum, and absorbed E6005 is rapidly metabolized to M11. Furthermore, the metabolite M11 is 100 times weaker than E6005 in suppressing cytokine production by human monocytes, possibly because of its lower cell membrane permeability (22). Thus, the adverse reactions commonly reported with the use of other PDE4 inhibitors are expected to be less common with topical E6005 use.

Application of E6005 ointment reduced the intensity of atopic dermatitis symptoms. The severity score of the targeted lesion was reduced in an E6005-concentration-dependent manner, with a significant reduction in the 0.2% E6005 treatment group (Figure 3-6). Patients receiving the 0.1% or 0.2% E6005 treatment had significantly greater improvement in most efficacy parameters than patients receiving the vehicle treatment. The reported minimal clinically important difference is 8.7/103

for the SCORAD index, 8.2/83 for the objective SCORAD index, and 6.6/72 for the EASI score (45). Therefore, the improvements seen in the 0.1% and 0.2% E6005 treatment groups were clinically important, and without safety concerns. 0.2% was considered as the clinically recommended dose for E6005.

The edema/papulation (swelling) and oozing/crust severity score of the target lesion showed an approximate 70-80% reduction in the 0.2% E6005 treatment groups, and the erythema (redness), excoriation (scratching), and lichenification scores showed a 30-50% reduction (Figure 3-6). The erythema (redness) and induration/papulation/ edema (thickness) EASI score showed a 50% reduction in the 0.2% E6005 treatment groups, and excoriation (scratching) and lichenification score showed a 20-40% reduction (Figure 3-7). These results suggest that 10-day application to a part of the body is not sufficient to evaluate the efficacy of E6005 ointment, and longer-term assessments with application to the entire body are required.

In mouse models, E6005 application has an immediate anti-pruritic effect via a cAMP-protein kinase A-dependent pathway (22, 24–26). Histamine binding to the H1 receptor does not affect the cAMP-protein kinase A-dependent pathway, but it does affect phospholipase C-dependent pathways, which results in allergic reaction hypersensitivity responses, including redness, itching, and swelling (46). PDE4 inhibitors do not affect histamine-induced itch, but may alter itching caused by other mechanisms such as serine proteases and proteinase-activated receptor 2 (26). In the present study, the subjective symptom score (pruritus and sleep loss) in the SCORAD index decreased (approximately 40% reduction), but did not disappear (Table 3-6, Figure 3-8). Concomitant use of histamine H1 receptor antagonists may be important to maximize the antipruritic effect of PDE4 inhibitors.

In summary, the present data indicate that E6005 ointment is safe and well tolerated and that it improves inflammation in patients with atopic dermatitis. However, the present results should be interpreted with caution because of the small study population, and also because only adult male patients were assessed during hospitalization, the drug was not applied to the entire body, and the treatment was short term. Longer-term efficacy studies with application of E6005 ointment to areas throughout the body are required.

Acknowledgement

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Table 3-1. Pruritus Score

Item	Description
Day	4. Very severe, interfering with daily activities 3. Severe, very annoying, substantially interfering with daily activities 2. Moderate, annoying and troublesome, may interfere with daily activities 1. Mild, not annoying or troublesome 0. None
Night	4. Very severe, interfering with sleep 3. Severe, very annoying, substantially interfering with sleep 2. Moderate, annoying and troublesome, may interfere with sleep 1. Mild, not annoying or interfering with sleep 0. None

Table 3-2. Demographics

Category	Vehicle (N=8)	E6005 0.01% (N=8)	E6005 0.03% (N=8)	E6005 0.1% (N=8)	E6005 0.2% (N=8)	E6005 Total (N=32)	Combined Total (N=40)
Age (year)[a]							
Mean (SD)	27.3 (8.6)	25.5 (6.8)	24.5 (3.8)	29.3 (7.5)	29.4 (9.2)	27.2 (7.1)	27.2 (7.3)
Median	25.0	22.0	23.5	28.5	26.5	24.0	24.0
Min, Max	20, 45	20, 40	20, 33	23, 46	20, 42	20, 46	20, 46
Height (cm)							
Mean (SD)	170.58 (8.04)	170.84 (4.85)	173.10 (5.77)	175.05 (4.85)	171.43 (5.20)	172.60 (5.20)	172.20 (5.81)
Median	169.90	171.35	173.70	175.05	171.15	172.45	171.40
Min, Max	161.7, 188.3	160.5, 177.1	163.3, 181.3	169.9, 184.2	162.1, 180.4	160.5, 184.2	160.5, 188.3
Weight (kg)							
Mean (SD)	66.53 (8.97)	68.08 (7.47)	62.95 (12.19)	67.28 (7.44)	65.74 (9.42)	66.01 (9.09)	66.11 (8.96)
Median	65.20	67.05	58.00	66.80	66.10	65.85	65.85
Min, Max	55.2, 76.9	54.7, 79.6	51.3, 85.3	55.2, 81.6	54.1, 78.7	51.3, 85.3	51.3, 85.3
BMI (kg/m^2)							
Mean (SD)	22.90 (3.11)	23.39 (2.85)	20.93 (3.39)	21.95 (2.37)	22.35 (2.89)	22.15 (2.90)	22.30 (2.91)
Median	22.05	22.55	19.75	21.55	22.35	22.10	22.10
Min, Max	20.1, 29.0	18.8, 27.8	18.3, 28.0	19.0, 26.6	18.6, 25.9	18.3, 28.0	18.3, 29.0

Percentages are based on the total number of subjects in relevant treatment group.
BMI: body mass index
a: Age is calculated at date of informed consent.

Table 3-3. Baseline characteristics

Category	Vehicle (N=8)	E6005 0.01% (N=8)	E6005 0.03% (N=8)	E6005 0.1% (N=8)	E6005 0.2% (N=8)	E6005 Total (N=32)	Combined Total (N=40)
Severity of atopic dermatitis, n (%)							
Mild	1 (12.5)	2 (25.0)	3 (37.5)	2 (25.0)	2 (25.0)	9 (28.1)	10 (25.0)
Moderate	5 (62.5)	3 (37.5)	5 (62.5)	4 (50.0)	5 (62.5)	17 (53.1)	22 (55.0)
Severe	1 (12.5)	2 (25.0)	0 (0.0)	2 (25.0)	1 (12.5)	5 (15.6)	6 (15.0)
Very Severe	1 (12.5)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)	2 (5.0)
Atopic dermatitis duration (years)							
Mean (SD)	11.30 (8.61)	17.71 (9.69)	22.73 (6.02)	16.55 (7.34)	21.01 (8.66)	19.50 (8.05)	17.86 (8.71)
Median	11.80	17.20	20.80	14.90	19.3	19.35	18.70
Min, Max	0.8, 24.8	5.8, 36.7	14.8, 34.8	8.9, 28.9	11.9, 35.9	5.8, 36.7	0.8, 36.7
Seasonal allergy, n (%)							
Absent	8 (100.0)	8 (100.0)	7 (87.5)	8 (100.0)	8 (100.0)	31 (96.9)	39 (97.5)
Present	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	1 (3.1)	1 (2.5)
Allergic rhinitis, n (%)							
Absent	7 (87.5)	6 (75.0)	7 (87.5)	7 (87.5)	7 (87.5)	27 (84.4)	34 (85.0)
Present	1 (12.5)	2 (25.0)	1 (12.5)	1 (12.5)	1 (12.5)	5 (15.6)	6 (15.0)
Allergic conjunctivitis, n (%)							
Absent	8 (100.0)	7 (87.5)	8 (100.0)	8 (100.0)	8 (100.0)	31 (96.9)	39 (97.5)
Present	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)	1 (2.5)
Severity score of targeted eczema							
Mean (SD)	6.6 (1.3)	9.1 (3.1)	6.3 (1.8)	5.8 (2.0)	7.0 (2.0)	7.0 (2.5)	7.0 (2.3)
Min, Max	5, 8	6, 15	4, 9	3, 8	5, 10	3, 15	3, 15
EASI score							
Mean (SD)	15.98 (8.56)	28.74 (17.19)	12.96 (6.25)	13.74 (9.70)	16.05 (7.03)	17.87 (12.24)	17.49 (11.53)
Min, Max	7.0, 32.0	8.2, 61.2	6.0, 25.0	1.4, 34.0	7.2, 28.0	1.4, 61.2	1.4, 61.2
SCORAD Total							
Mean (SD)	43.04 (6.00)	52.55 (20.61)	40.89 (11.49)	38.14 (11.79)	41.48 (13.04)	43.26 (15.06)	43.22 (13.67)
Min, Max	31.2, 52.5	24.0, 88.5	24.6, 56.2	23.1, 61.4	23.5, 56.8	23.1, 88.5	23.1, 88.5
SCORAD-A							
Mean (SD)	54.4 (26.2)	66.0 (31.8)	44.1 (30.9)	42.1 (28.1)	36.3 (16.6)	47.1 (28.6)	48.6 (28.0)
Min, Max	25, 90	15, 95	10, 95	10, 92	15, 70	10, 95	10, 95
SCORAD-B							
Mean (SD)	7.0 (1.4)	9.0 (4.2)	6.9 (2.0)	6.8 (1.8)	8.1 (2.2)	7.7 (2.8)	7.6 (2.6)
Min, Max	5, 9	4, 17	4, 10	5, 10	5, 11	4, 17	4, 17
Objective SCORAD							
Mean (SD)	35.38 (7.75)	44.70 (18.83)	32.89 (8.81)	32.05 (10.69)	35.69 (10.14)	36.33 (13.14)	36.14 (12.17)
Min, Max	23.5, 48.5	20.0, 78.5	20.0, 42.1	19.5, 53.4	20.5, 46.5	19.5, 78.5	19.5, 78.5
SCORAD-C							
Mean (SD)	7.66 (3.41)	7.85 (3.01)	8.00 (4.42)	6.09 (1.96)	5.79 (3.94)	6.93 (3.44)	7.08 (3.41)
Min, Max	2.4, 12.9	3.7, 12.6	3.0, 14.1	3.6, 8.9	2.4, 13.8	2.4, 14.1	2.4, 14.1
Pruritus Score (day)							
Mean (SD)	2.0 (0.0)	2.5 (0.5)	2.3 (0.7)	2.0 (0.0)	2.4 (0.5)	2.3 (0.5)	2.2 (0.5)
Min, Max	2, 2	2, 3	1, 3	2, 2	2, 3	1, 3	1, 3
Pruritus Score (night)							
Mean (SD)	1.9 (0.4)	1.8 (0.5)	2.0 (0.8)	1.9 (0.6)	1.9 (0.6)	1.9 (0.6)	1.9 (0.6)
Min, Max	1, 2	1, 2	1, 3	1, 3	1, 3	1, 3	1, 3

Percentages are based on the total number of subjects in relevant treatment group.
EASI: Eczema Area and Severity Index, EQ-5D: the EuroQol Group 5-Dimension Self-Report Questionnaire score, IgE: immunoglobulin E, LDH: lactic dehydrogenase, SCORAD: SCORing Atopic Dermatitis, CCL17: thymus and activation-regulated chemokine (TARC), TEWL: transepidermal water loss.

Table 3-3. Baseline characteristics (continued)

Category	Vehicle (N=8)	E6005 0.01% (N=8)	E6005 0.03% (N=8)	E6005 0.1% (N=8)	E6005 0.2% (N=8)	E6005 Total (N=32)	Combined Total (N=40)
Targeted eczema area TEWL (g/m^2h)							
Mean (SD)	30.15 (12.66)	23.19 (5.01)	40.64 (17.16)	28.81 (12.14)	24.18 (4.83)	29.20 (12.66)	29.39 (12.50)
Min, Max	12.6, 56.2	13.2, 28.2	22.3, 68.1	14.8, 47.0	18.0, 32.7	13.2, 68.1	12.6, 68.1
Non-eczema area TEWL (g/m^2h)							
Mean (SD)	16.11 (5.92)	16.38 (6.06)	24.23 (7.56)	12.51 (3.79)	15.59 (3.26)	17.18 (6.79)	16.96 (6.57)
Min, Max	10.1, 26.6	9.6, 25.7	13.9, 37.2	8.5, 20.8	11.5, 22.2	8.5, 37.2	8.5, 37.2
Eosinophil counts (/μL)							
Mean (SD)	351.5 (214.6)	1065.0 (1075.3)	485.8 (328.2)	539.8 (324.7)	379.0 (181.1)	617.4 (623.7)	564.2 (573.7)
Median	309.0	632.0	504.5	450.0	323.5	454.5	439.5
Min, Max	89, 704	404, 3578	99, 1006	188, 1024	240, 794	99, 3578	89, 3578
LDH (IU/L)							
Mean (SD)	174.9 (30.2)	270.1 (146.1)	227.1 (95.1)	195.6 (48.9)	196.5 (29.1)	222.3 (92.4)	212.9 (85.6)
Median	182.5	236.0	191.0	185.0	186.5	194.0	187.5
Min, Max	113, 205	153, 602	152, 446	132, 270	171, 257	132, 602	113, 602
IgE (IU/mL)							
Median	290.0	1950.0	395.0	735.0	2350.0	980.0	735.0
Min, Max	39, 23000	30, 25000	9, 5400	170, 4200	23, 7700	9, 25000	9, 25000
CCL17 (pg/mL)							
Median	374.5	812.0	629.5	489.0	372.5	543.0	507.0
Min, Max	302, 4010	507, 48000	206, 3040	146, 948	172, 1460	146, 48000	146, 48000
EQ-5D							
Mean (SD)	0.9341 (0.1231)	0.9420 (0.1074)	0.9710 (0.0820)	1.0000 (0.0000)	0.9341 (0.1231)	0.9618 (0.0908)	0.9563 (0.0970)
Median	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Min, Max	0.705, 1.000	0.768, 1.000	0.768, 1.000	1.000, 1.000	0.705, 1.000	0.705, 1.000	0.705, 1.000
Skindex-16 Total Score							
Mean (SD)	36.3 (19.2)	45.1 (13.8)	42.5 (22.7)	29.1 (11.6)	33.8 (24.6)	37.6 (19.2)	37.4 (19.0)
Median	36.5	49.0	38.5	26.0	27.5	31.5	34.0
Min, Max	7, 70	19, 58	12, 72	18, 54	4, 84	4, 84	4, 84

Percentages are based on the total number of subjects in relevant treatment group.
EASI: Eczema Area and Severity Index, EQ-5D: the EuroQol Group 5-Dimension Self-Report Questionnaire score, IgE: immunoglobulin E, LDH: lactic dehydrogenase, SCORAD: SCORing Atopic Dermatitis, CCL17: thymus and activation-regulated chemokine (TARC), TEWL: transepidermal water loss.

Table 3-4. Treatment-emergent adverse events

MedDRA System organ class and preferred term [a]	Vehicle	E6005			
		0.01%	0.03%	0.1%	0.2%
	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)
Any adverse event	1 (12.5%)	0	1 (12.5%)	0	1 (12.5%)
Gastrointestinal disorders					
Enterocolitis	0	0	0	0	1 (12.5%)
Infections and infestations					
Kaposi's varicelliform eruption	1 (12.5%)	0	0	0	0
Metabolism and nutrition disorders					
Gout	0	0	1 (12.5%)	0	0
Skin and subcutaneous tissue disorders					
Dermatitis, atopic	1 (12.5%)	0	0	0	0

a: Medical Dictionary for Regulatory Activities, version 17.1

Table 3-5. Number of subjects with plasma concentrations of E6005 or M11 above the lower limit of quantification

	Vehicle	E6005			
		0.01%	0.03%	0.1%	0.2%
	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)
E6005	0	0	0	0	0
M11	0	0	0	2 (25.0%)	1 (12.5%)

The lower limit of quantification was 1.00 ng/mL

Table 3-6. Mean changes in efficacy variables from baseline through Day 11

Variable	Vehicle	E6005			
		0.01%	0.03%	0.1%	0.2%
	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)
Severity score, targeted lesion					
Day 1 (baseline), mean±SD	6.6±1.3	9.1±3.1	6.3±1.8	5.8±2.0	7.0±2.0
Day 11(LOCF), mean±SD	6.0±2.5	7.8±3.2	3.9±1.8	3.9±2.5	3.3±1.7
Mean change±SD	-0.6±1.8	-1.4±2.3	-2.4±0.5	-1.9±2.4	-3.8±1.4
95% CI of difference from vehicle [a]	—	-2.2, 1.7	-3.6, 0.0	-3.3, 0.4	-4.8, -1.2
p-value [a]	—	0.825	0.046	0.117	0.001
Mean percent change±SD	-11.39±33.03	-14.48±24.97	-41.06±16.50	-28.38±47.16	-54.30±17.44
95% CI of difference from vehicle [a]	—	-37.41, 29.31	-60.56, 1.50	-47.92, 14.62	-74.08, -12.03
p-value [a]	—	0.806	0.061	0.286	0.007
EASI score					
Day 1 (baseline), mean±SD					
Head and neck	0.90±0.77	2.23±2.33	0.53±0.68	1.49±0.89	1.84±0.77
Trunk	6.83±3.07	10.24±6.12	5.59±2.33	6.08±3.66	5.70±2.21
Upper limbs	2.90±1.44	5.18±3.45	2.05±1.62	2.58±2.42	3.35±2.08
Lower limbs	5.35±3.92	11.10±6.36	4.80±2.47	3.60±3.78	5.20±4.82
EASI score total	15.98±8.56	28.74±16.39	10.99±5.56	12.51±10.43	13.51±4.45
Day 11, mean±SD					
Head and neck	0.80±0.66	2.01±1.78	0.41±0.50	0.98±0.46	1.05±0.50
Trunk	5.96±3.46	9.49±5.48	4.07±1.95	2.48±1.57	3.30±1.50
Upper limbs	2.66±1.81	4.65±2.62	1.89±1.21	0.95±0.82	2.08±1.57
Lower limbs	4.86±3.98	9.55±4.49	3.60±2.08	2.20±3.06	3.50±2.94
EASI score total	14.27±9.25	25.70±12.26	9.97±5.18	6.60±4.17	9.93±5.69
EASI score total, mean change±SD	-2.31±2.12	-3.04±7.54	-3.37±4.08	-7.14±6.15	-6.13±2.72
95% CI of difference from vehicle [b]	—	-2.58, 5.71	-6.17, 2.03	-9.77, -1.82	-8.24, -0.31
p-value [b]	—	0.452	0.317	0.004	0.035
SCORAD index					
Day 1 (baseline), mean±SD					
Objective SCORAD	35.38±7.75	44.70±18.83	32.89±8.81	32.05±10.69	35.69±10.14
SCORAD-C	7.66±3.41	7.85±3.01	8.00±4.42	6.09±1.96	5.79±3.94
SCORAD index total	43.04±6.00	52.55±20.61	40.89±11.49	38.14±11.79	41.48±13.04
Day 11, mean±SD					
Objective SCORAD	33.07±11.16	43.56±14.98	27.01±9.11	23.06±6.11	28.69±10.66
SCORAD-C	5.84±3.56	6.70±3.96	3.69±2.00	3.68±2.40	3.73±2.15
SCORAD index total	38.91±11.00	50.26±17.31	30.70±9.24	26.74±7.09	32.41±11.52
SCORAD total, mean change±SD	-4.20±5.11	-2.29±9.25	-9.47±7.26	-11.40±10.91	-9.06±7.20
95% CI of difference from vehicle [b]	—	-4.66, 10.50	-14.45, 0.90	-16.79, -1.82	-13.70, 1.21
p-value [b]	—	0.444	0.082	0.015	0.098
Pruritus Score					
Day					
Day 1 (baseline), mean±SD	2.0±0.0	2.5±0.5	2.3±0.7	2.0±0.0	2.4±0.5
Day 11 (LOCF), mean±SD	2.3±0.7	1.9±0.6	1.6±0.5	1.9±0.4	1.8±0.9
Mean change from baseline±SD	0.3±0.7	-0.6±0.7	-0.6±0.7	-0.1±0.4	-0.6±1.1
95% CI of difference from vehicle	—	-1.7, -0.1	-1.7, -0.1	-1.0, 0.2	-1.8, 0.1
Night					
Day 1 (baseline), mean±SD	1.9±0.4	1.8±0.5	2.0±0.8	1.9±0.6	1.9±0.6
Day 11 (LOCF), mean±SD	2.0±0.9	2.0±0.8	1.5±0.5	1.4±0.9	1.5±0.8
Mean change from baseline±SD	0.1±0.8	0.3±0.7	-0.5±0.9	-0.5±0.8	-0.4±1.2
95% CI of difference from vehicle	—	-0.7, 1.0	-1.6, 0.3	-1.5, 0.2	-1.6, 0.6

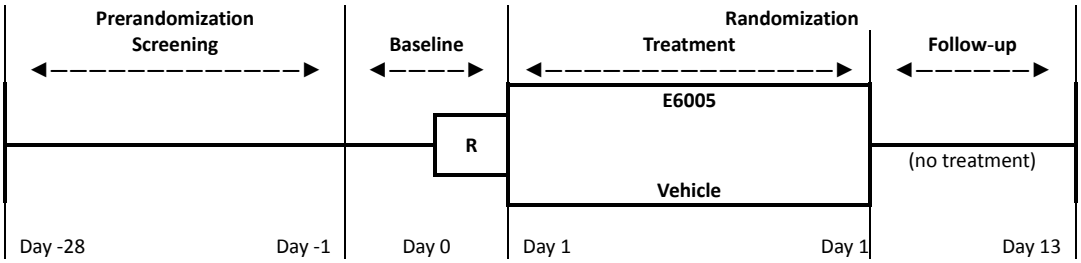
CI: confidence interval, EASI: eczema area and severity index, LOCF: last observation carried forward, SCORAD:scoring atopic dermatitis, SD: standard deviation.
a: *p*-values and 95% CIs were obtained from analysis of covariance with treatment as a factor and baseline scores as a covariate.
b: *p*-values and 95% CIs were obtained from linear mixed-effect model with treatment and time as factors, baseline score as a covariate, subject as random effect, and changes or percent changes from baseline to days 5 and 11 as repeated measurements

Table 3-6. Mean changes in efficacy variables from baseline through Day 11

(continued)

Variable	Vehicle	E6005			
		0.01%	0.03%	0.1%	0.2%
	(n=8)	(n=8)	(n=8)	(n=8)	(n=8)
TEWL (g/m^2h), targeted eczema					
Day 1 (baseline), mean±SD	30.15±12.66	23.19±5.01	40.64±17.16	28.81±12.14	24.18±4.83
Day 11 (LOCF), mean±SD	27.36±12.94	24.11±7.17	30.29±7.21	20.91±4.73	19.18±5.45
Mean change from baseline±SD	-2.79±12.45	0.93±3.39	-10.35±12.71	-7.90±10.71	-5.00±5.47
95% CI of difference from vehicle	—	-6.07, 13.49	-21.05, 5.92	17.56, 7.34	-12.52, 8.10
Eosinophile counts (/μL)					
Day 1 (baseline), mean±SD	351.5±214.6	1065.0±1075.3	485.8±328.2	539.8±324.7	379.0±181.1
Day 11 (LOCF), mean±SD	312.6±234.5	745.0±731.2	301.0±228.1	441.6±312.3	275.3±134.5
Mean change from baseline±SD	312.6±234.5	745.0±731.2	301.0±228.1	441.6±312.3	275.3±134.5
95% CI of difference from vehicle	—	-585.1, 22.9	-376.9, 85.1	-188.2, 69.7	-184.0, 54.3
LDH (IU/L)					
Day 1 (baseline), mean±SD	174.9±30.2	270.1±146.1	227.1±95.1	195.6±48.9	196.5±29.1
Day 11 (LOCF), mean±SD	171.9±73.0	212.4±108.4	170.6±45.5	171.5±40.1	172.3±23.0
Mean change from baseline±SD	-3.0±59.8	-57.8±39.8	-56.5±65.3	-24.1±19.4	-24.3±16.2
95% CI of difference from vehicle	—	-109.2, -0.3	-120.7, 13.7	-68.8, 26.5	-68.2, 25.7
Total IgE (IU/mL)					
Day 1 (baseline), mean±SD	3441.5±7943.5	5835.0±8608.0	1154.1±1814.3	1335.0±1415.9	2491.0±(2575.6
Day 11 (LOCF), mean±SD	3466.4±7939.8	5459.3±7433.3	1119.8±1623.7	1360.0±1476.8	2267.6±2316.0
Mean change from baseline±SD	24.9±71.6	-375.8±1554.3	-34.4±239.5	25.0±112.4	-223.4±342.5
95% CI of difference from vehicle	—	-1580.5, 779.3	-248.8, 130.3	-100.9, 101.2	-513.5, 17.0
CCL17 (pg/mL)					
Day 1 (baseline), mean±SD	869.5±1277.5	6832.6±16644.9	918.9±963.6	478.6±252.3	627.0±505.4
Day 11 (LOCF), mean±SD	1927.8±4313.8	3001.3±6014.5	842.5±1025.0	481.9±187.4	519.3±378.4
Mean change from baseline±SD	1058.3±3043.8	-3831.4±10655.4	-76.4±310.2	3.3±177.6	-107.8±235.9
95% CI of difference from vehicle	—	-13292.7, 3513.5	-3454.7, 1185.4	-3367.0, 1257.0	-3481.0, 1149.0

CCL17: thymus and activation-regulated chemokine (TARC), CI: confidence interval, EASI: eczema area and severity index, LOCF: last observation carried forward, SCORAD: scoring atopic dermatitis, SD: standard deviation.
a: *p*-values and 95% CIs were obtained from analysis of covariance with treatment as a factor and baseline scores as a covariate.
b: *p*-values and 95% CIs were obtained from linear mixed-effect model with treatment and time as factors, baseline score as a covariate, subject as random effect, and changes or percent changes from baseline to days 5 and 11 as repeated measurements



R: Randomization

Figure 3-1. Study design for E6005-J081-101 (shown as a cohort)

Row		Head and Neck	Upper Limbs	Trunk	Lower Limbs
1	Erythema	E	E	E	E
2	Induration/Papulation	I			
3	Excoriations	Ex			
4	Lichenification	L	L	L	L
5	Total each Column	E+I+Ex+L	E+I		
6	Area	Area			
7	Multiply Row 5 by Row 6	(E+I+Ex+L) x Area	(E+I x		
8		(E+I+Ex+L) x Area x 0.10	(E+I+Ex+L) x Area x 0.20	(E+I+Ex+L) x Area x 0.30	(E+I+Ex+L) x Area x 0.40
9	Multiply row 7 by Row 8	H	UL	T	LL
10	Total EASI (add together each column from Row 9)	H+UL+T+LL			

Rank severity of atopic lesions:
0= none, 1= mild, 2=moderate, 3= severe

Rank area of atopic involvement:
0= none, 1= <10%, 2=10% to <30%,
3= 30% to <50%, 4=50% to <70%,
5= 70% to <90%, 6=90% to 100%

Figure 3-2. EASI score

The body was divided into four anatomic regions (head and neck, upper limbs, trunk, and lower limbs), and the items described below were assessed. It should be noted that the buttocks and feet were counted as part of the lower limbs, the internal axillae and groin were counted as part of the trunk, and the external axillae and hands were counted as part of the upper limbs.

Area: The area within each body region with the key signs of inflammation was calculated as the percentage of the total area of the body region based on seven classifications (0: 0%, 1: 1 to 9%, 2: 10 to 29%, 3: 30 to 49%, 4: 50 to 69%, 5: 70 to 89%, 6: 90 to 100%). Symptoms (e.g., pruritus), along with secondary signs (e.g.,

xerosis and scaling) was excluded from the area assessments.

Symptoms: Each of the four body regions was assessed separately for the key signs of erythema (E), infiltration and/or papulation (I), excoriations (Ex), and lichenification (L). The average degree of severity of each sign in each of the four body regions was assessed based on the four classifications (0: none, 1: mild, 2: moderate, and 3: severe). The score was calculated for each region [(E+I+Ex+L) × Area], and then multiplied the head and neck score by 0.1, the upper limbs score by 0.2, the trunk score by 0.3, and the lower limbs score by 0.4. The Eczema Area and Severity Index (EASI) score (0 – 72) was obtained by summing the four scores calculated above.

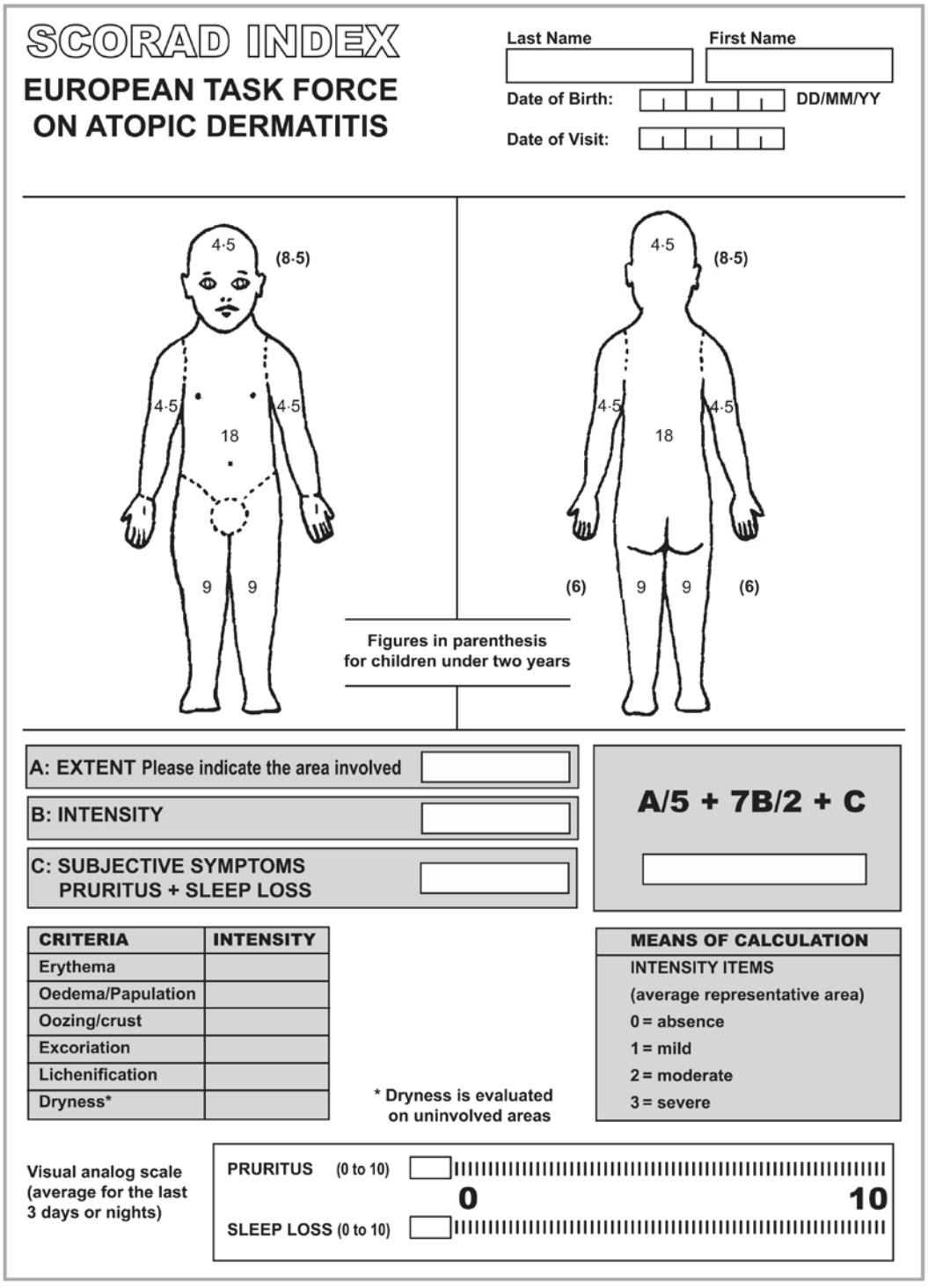


Figure 3-3. SCORAD index

At each point of assessment, the percentage of eczema area to the entire body (area ratio, %) was calculated according to the area ratio in the assessment procedure of

SCORAD. This was reported as SCORAD-A (extent of eczema).

At each point of assessment, the investigators divided the symptoms into five items (erythema, oedema/papulation, oozing/crust, excoriation, and lichenification) according to the assessment procedure of SCORAD, selected the average area for each item, assessed the severity based on four classifications (0: none, 1: mild, 2: moderate, and 3: severe). The dryness of the skin in non-eczema area was assessed based on four classifications (0: none, 1: mild, 2: moderate, and 3: severe). The sum of the six items was calculated to present SCORAD-B (intensity of eczema).

Subjects evaluated the degree of “Pruritus” and “Sleep loss” associated with atopic dermatitis as an average over the 3 days before the assessments and filled in the 10 cm visual analogue scale in handwriting. Documentation of the results was included in the source documentation at the investigational site. This evaluation was conducted before all the examinations and assessments by the investigators, in order to avoid influences on the assessment of the subject. The investigators and clinical research coordinators checked the reported score to see if there were no inadequacy or inconsistency, measured the length from far left (cm) to the first decimal place. The sum of the two items was calculated the to produce SCORAD-C (subjective symptoms).

The objective SCORAD score (0 – 83) and the SCORAD score (0 – 103) were calculated using the following formulae.

Objective SCORAD = SCORAD-A / 5 + SCORAD-B × 7 / 2

SCORAD = Objective SCORAD + SCORAD-C

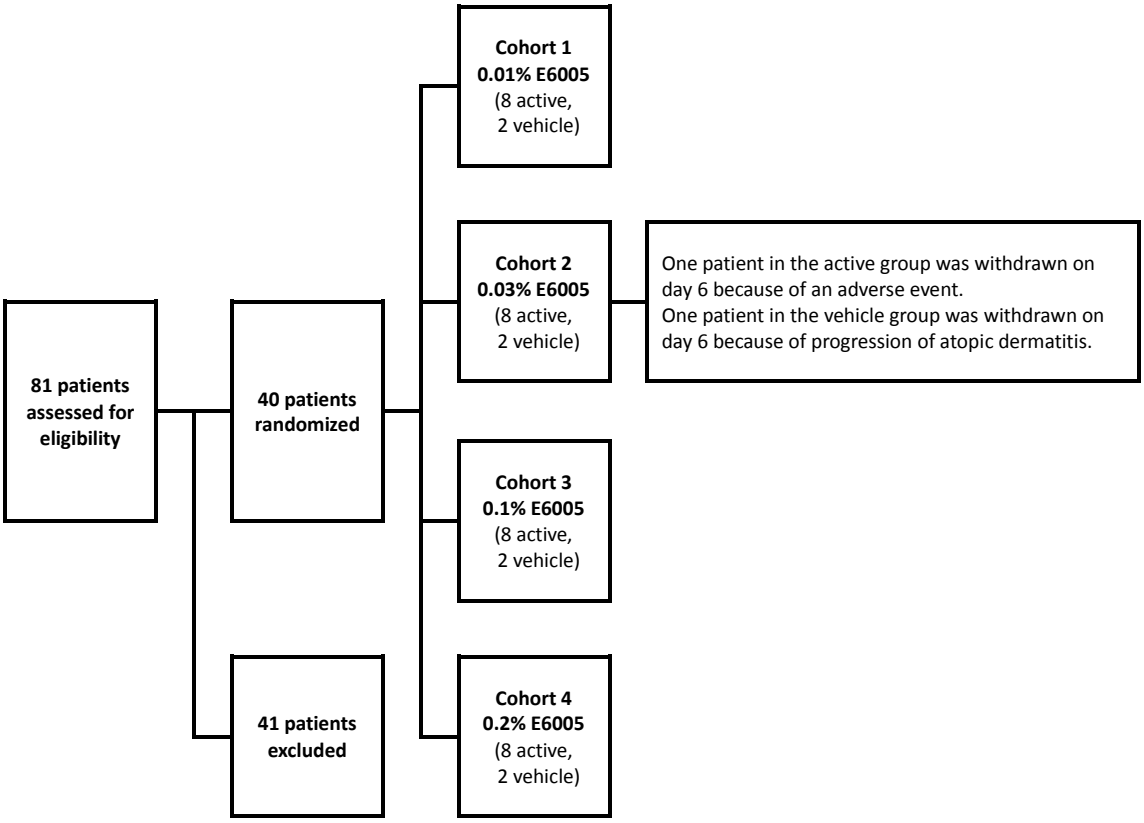


Figure 3-4. Flow chart of patients included in E6005-J081-101

Of the 81 patients screened, 40 who had typically eczematous skin lesions that were on their posterior trunk that could be evaluated were randomized into four groups of 10. Patients in each group were randomized (at a ratio of 4:1) to receive application of either 5 g of E6005 ointment or 5 g of vehicle ointment. Investigators and patients were blinded to application of the study drug. When no safety concerns arose within a cohort, the next cohort began treatment at a higher drug concentration.

A B

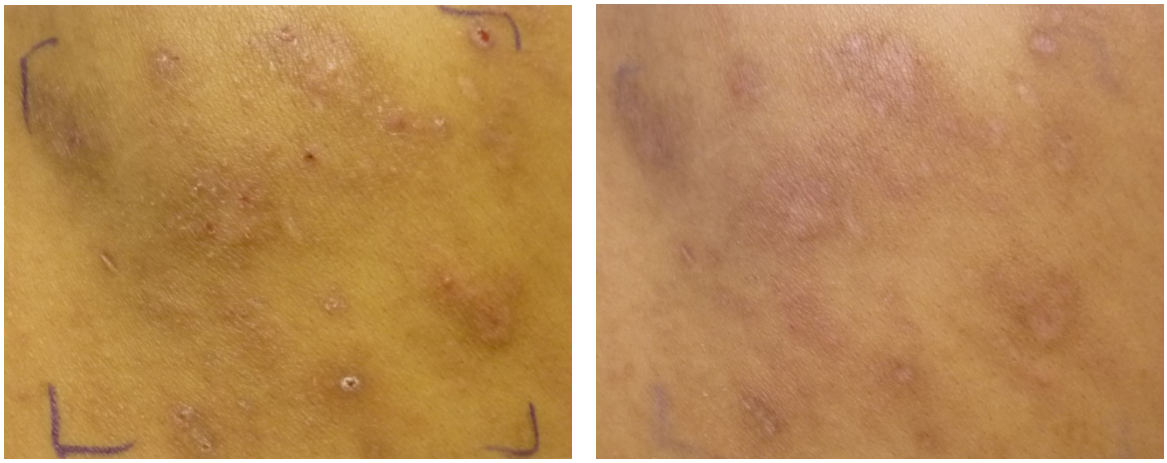


Figure 3-5. Photographs of a targeted lesion in a 20-year-old participant treated with 0.2% E6005 ointment.

(A) Severity score at baseline was 9/15. Application of E6005 ointment reduced the intensity of edema/papulation, oozing/crust, excoriation, and lichenification, but not erythema.

(B) Severity score on day 11 had fallen to 5/15.

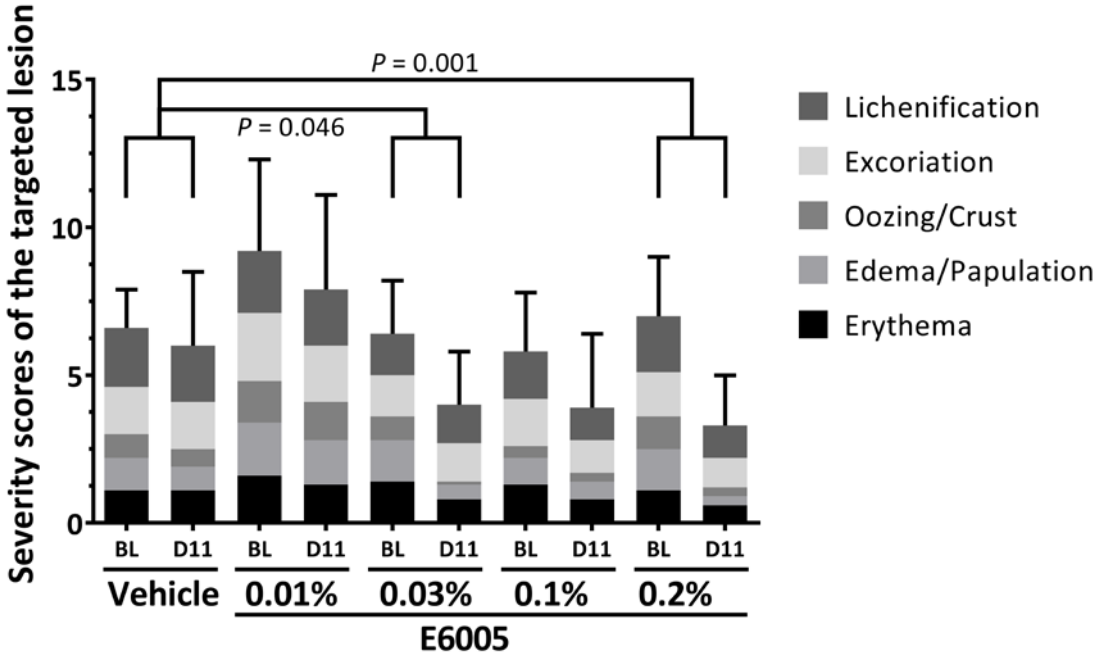


Figure 3-6. Severity score of targeted lesions.

The severity score (range: 0−15) of the targeted lesion was calculated as the sum of intensity scores (0: none, 1: mild, 2: moderate, and 3: severe) for each of five symptoms (erythema, edema and/or papulation, oozing and/or crust, excoriation, lichenification), according to the SCORAD index assessment procedure. Differences in the severity scores (last observation carried forward) were evaluated using ANCOVA, with treatment as a factor and baseline score as a covariate. Error bars show standard deviation.

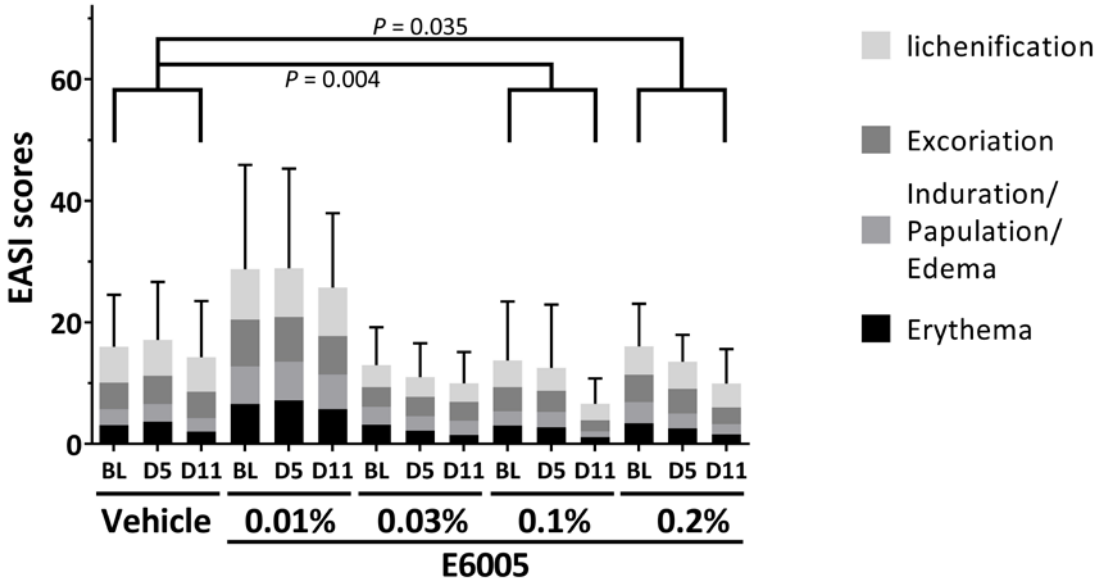


Figure 3-7. EASI score.

Differences in the EASI score (observed case) were analyzed using a linear mixed-effect model, with treatment and time as factors, baseline score as a covariate, subject as random effect, and changes from baseline as repeated measurements.

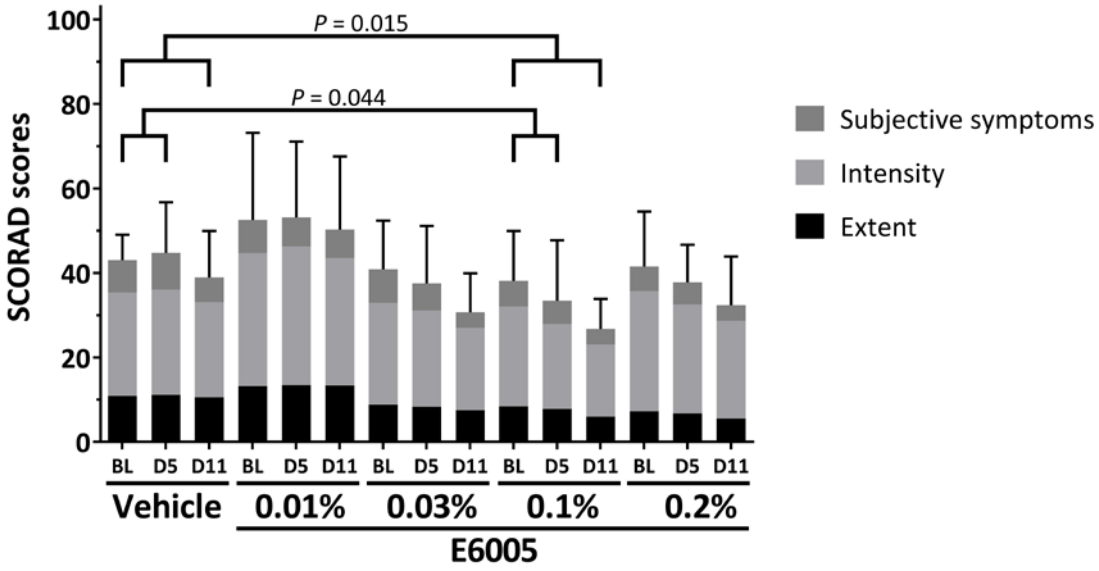


Figure 3-8. SCORAD index.

Differences in the SCORAD index (observed case) were analyzed using a linear mixed-effect model, with treatment and time as factors, baseline score as a covariate, subject as random effect, and changes from baseline as repeated measurements.

Chapter 4.

Concluding Remarks

Atopic dermatitis is a common skin disorder (1). Individuals with atopic dermatitis have increased phosphodiesterase activity in their white blood cells, which leads to decreased cyclic AMP levels and loss of cAMP's anti-inflammatory effects (14, 17–19). For years, there has been interest in the use of phosphodiesterase inhibitors to treat chronic inflammatory diseases. However, the use of these drugs has been limited because of their systemic side effects (20, 21). E6005 is a novel phosphodiesterase inhibitor developed for topical use. Topical E6005 has shown anti-inflammatory and anti-pruritic effects in mouse models (22–26).

I conduct two studies, one is to evaluate the safety and pharmacokinetics of E6005 ointment in healthy volunteers and another is to evaluate the safety, pharmacokinetics and efficacy of E6005 ointment in patients with atopic dermatitis (27, 28).

Thirty-eight healthy volunteers and 40 patients with atopic dermatitis participate in these randomized, investigator-blind, vehicle-controlled studies. The 38 healthy volunteers undergo skin patch testing, photosensitivity testing, increasing dose testing, and repeated twice-daily dosing. The 40 patients with atopic dermatitis are divided into four cohorts, each of which underwent a 10-day course of topical treatment of lesions at a different drug concentration. The safety profile of E6005 is assessed with treatment-emergent adverse event reporting, clinical laboratory testing,

electrocardiogram recording, vital signs, and ophthalmological findings. E6005 is found to be as safe and well tolerated as petrolatum ointment, and plasma concentrations of the drug were below detectable levels in all participants. None of the participants experiences the most common side effects of systemic PDE4 inhibitor administration: nausea, vomiting, or headache.

Additionally, I investigate the efficacy of a 10-day course of topical E6005 ointment at four different concentrations, using vehicle ointment as a control. Efficacy is evaluated based on changes in the severity scores of targeted lesions, SORAD indexes, EASI scores, and laboratory parameters. Severity scores decreased in a concentration-dependent manner in the E6005 treatment groups, with a significant improvement in the 0.2% E6005 group compared with the vehicle group.

Recently, Furue M, *et al.* have reported E6005 treatment is safe and effective in adults with atopic dermatitis (47) and Nemoto O, *et al.* have reported E6005 treatment is safe and effective in children with atopic dermatitis (48). These results suggest that E6005 ointment can provide a new treatment option for atopic dermatitis that avoids safety concerns associated with PDE4 inhibitors, while maintaining similar effectiveness.

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References

1. Darsow U, Wollenberg A, Simon D, Taïeb A, Werfel T, Oranje A, Gelmetti C, Svensson A, Deleuran M, Calza AM, Giusti F, Lübbe J, Seidenari S, Ring J; European Task Force on Atopic Dermatitis/EADV Eczema Task Force. (2010) ETFAD/EADV eczema task force 2009 position paper on diagnosis and treatment of atopic dermatitis. *J Eur Acad Dermatol Venereol.* **24**:317-28.
2. Vinding GR, Zarchi K, Ibler KS, Miller IM, Ellervik C, Jemec GB. (2014) Is adult atopic eczema more common than we think? - A population-based study in Danish adults. *Acta Derm Venereol.* **94**:480-2.
3. Williams H, Stewart A, von Mutius E, Cookson W, Anderson HR; International Study of Asthma and Allergies in Childhood (ISAAC) Phase One and Three Study Groups. (2008) Is eczema really on the increase worldwide? *J Allergy Clin Immunol.* **121**:947-54.
4. Suzuki T, Kanzaki T. (Eds.) (2008) *Color atlas of dermatology. Vol. 1 Allergic skin diseases.* Tokyo:Kodansha. (in **Japanese**)
5. Hahn EL, Bacharier LB. (2005) The atopic march: the pattern of allergic disease development in childhood. *Immunol Allergy Clin N Am.* **25**:231-46.
6. Hanifin JM, Rajka G. (1980) Diagnostic features of atopic dermatitis. *Acta Derm Venereol.* **92**:44-7.
7. Akdis CA, Akdis M, Bieber T, Bindslev-Jensen C, Boguniewicz M, Eigenmann P, Hamid Q, Kapp A, Leung DY, Lipozencic J, Luger TA, Muraro A, Novak N, Platts-Mills TA, Rosenwasser L, Scheynius A, Simons FE, Spergel J, Turjanmaa K, Wahn U, Weidinger S, Werfel T, Zuberbier T; European Academy of Allergology;

Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Group. (2006) Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/ American Academy of Allergy, Asthma and Immunology/ PRACTALL Consensus Report. *J Allergy Clin Immunol.* **118**:152-69.

8. Furue M, Saeki H, Furukawa F, Hide M, Ohtsuki M, Nakamura T, Sasaki R, Suto H, Takehara K, Committee for Guidelines for Management of Atopic Dermatitis of Japanese Dermatological Association. (2008) Guideline for Management of Atopic Dermatitis. *Jpn J Dermatol.* **118**:325-42. (in **Japanese**)
9. Garmhausen D, Hagemann T, Bieber T, Dimitriou I, Fimmers R, Diepgen T, Novak N. (2013) Characterization of different courses of atopic dermatitis in adolescent and adult patients. *Allergy.* **68**:498-506.
10. Simon D, Bieber T. (2014) Systemic therapy for atopic dermatitis. *Allergy.* **69**:46-55.
11. Zuberbier T, Orlow S, Paller A, Taïeb A, Allen R, Hernanz-Hermosa JM, Ocampo-Candiani J, Cox M, Langeraar J, Simon JC. (2006) Patient perspectives on the management of atopic dermatitis. *J Allergy Clin Immunol.* **118**:226-32.
12. Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ. (2007) *Fitzpatrick's Dermatology in General Medicine, 7th ed.* New York,NY: Mcgraw-Hill.
13. Tamaki K. (2002) *Comprehensive handbook of clinical dermatology. Vol. 3 Eczema, prurigo, pruritus, erythroderma, urticaria.* Tokyo:Nakayama Shoten. (in **Japanese**)
14. Parker CW, Kennedy S, Eisen AZ. (1977) Leukocyte and lymphocyte cyclic AMP

responses in atopic eczema. *J Invest Dermatol.* **68**:302-6.

15. Abrahamsen H, Baillie G, Ngai J, Vang T, Nika K, Ruppelt A, Mustelin T, Zaccolo M, Houslay M, Taskén K. (2004) TCR- and CD28-Mediated Recruitment of Phosphodiesterase 4 to Lipid Rafts Potentiates TCR Signaling. *J. Immunol.* **173**:4847-58.
16. Jin SLC, Lan L, Zoudilova M, Conti M. (2005) Specific Role of Phosphodiesterase 4B in Lipopolysacchride-Induced Signaling in Mouse Macrophages. *J. Immunol.* **175**:1523-31.
17. Grewe SR, Chan SC, Hanifin JM. (1982) Elevated leukocyte cyclic AMP-phosphodiesterase in atopic disease: A possible mechanism for cyclic AMP-agonist hyporesponsiveness. *J Allergy Clin Immunol.* **70**:452-7.
18. Chan SC, Reifsnyder D, Beavo JA, Hanifin JM. (1993) Immunochemical characterization of the distinct monocyte cyclic AMP-phosphodiesterase from patients with atopic dermatitis. *J Allergy Clin Immunol.* **91**:1179-88.
19. Hanifin JM, Chan SC. (1995) Monocyte phosphodiesterase abnormalities and dysregulation of lymphocyte function in atopic dermatitis. *J Allergy Clin Immunol.* **105**(suppl):84S-88S.
20. Galvaldá A, Roberts RS. (2013) Phosphoduesterase-4 inhibitors: a review of current developments (2010-2012). *Expert Opin Ther Patents.* **23**:997-1016.
21. Pagés L, Gavaldá A, Lehner MD. (2009) PDE4 inhibitors: a review of current developments (2005-2009). *Expert Opin Ther Patents.* **19**:1501-19.
22. Ishii N, Shirato M, Wakita H, Miyazaki K, Takase Y, Asano O, Kusano K, Yamamoto E, Inoue C, Hishinuma I. (2013) Anti-pruritic effect of the topical phosphodiesterase 4 inhibitor E6005 ameliorates skin lesions in a mouse atopic

dermatitis model. *J Pharmacol Exp Ther*. **346**:105-12.

23. Ishii N, Wakita H, Shirato M. (2014) Effect of the phosphodiesterase 4 inhibitor E6005 on nerve growth factor elevation in irritated skin of NC/Nga mice. *J Dermatol Sci.* **76**:263-4.
24. Wakita H, Ohkuro M, Ishii N, Hishinuma I, Shirato M. (2015) A putative antipruritic mechanism of the phosphodiesterase-4 inhibitor E6005 by attenuating capsaicin-induced depolarization of C-fibre nerves. *Exp Dermatol.* **24**:215-6.
25. Andoh T, Yoshida T, Kuraishi Y. (2014) Topical E6005, a novel phosphodiesterase 4 inhibitor, attenuates spontaneous itch-related responses in mice with chronic atopy-like dermatitis. *Exp Dermatol.* **23**:359-61.
26. Andoh T, Kuraishi Y. (2014) Antipruritic mechanisms of topical E6005, a phosphodiesterase 4 inhibitor: Inhibition of responses to proteinase-activated receptor 2 stimulation mediated by increase in intracellular cyclic AMP. *J Dermatol Sci.* **76**:206-13.
27. Ohba F, Nomoto M, Hojo S, Akama H. (2016) Safety, tolerability and pharmacokinetics of a novel phosphodiesterase inhibitor, E6005 ointment, in healthy volunteers and in patients with atopic dermatitis. *J Dermatolog Treat.* **27**:241-6.
28. Ohba F, Matsuki S, Imayama S, Matsuguma K, Hojo S, Nomoto M, Akama H. (2016) Efficacy of a novel phosphodiesterase inhibitor, E6005, in patients with atopic dermatitis: An investigator-blinded, vehicle-controlled study. *J Dermatolog Treat.* **in press**.
29. Bieber T. (2013) Atopic dermatitis 2.0: from the clinical phenotype to the molecular taxonomy and stratified medicine. *Allergy.* **67**:1475-82.

30. Calverley PMA, Sanchez-Toril F, McIvor A, Teichmann P, Bredenbroeker D, Fabbri LM. (2007) Effect of 1-year treatment with roflumilast in severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* **176**:154-61.
31. Papp KA, Kaufmann R, Thaçhi D, Hu C, Sutherland D, Rohane P. (2013) Efficacy and safety of apremilast in subjects with moderate to severe plaque psoriasis: results from a phase II, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-comparison study. *J Eur Acad Dermatol Venereol.* **27**:e376-e383.
32. Wahlberg JE, Lindberg M. (2006) Patch testing. In Frosch PJ, Menne T, Lepoittevin JP. (Eds.) *Contact Dermatitis. 4th ed.* Berlin:Springer. pp. 366-90.
33. Irie S, Tsukikawa H, Nabeshima T, Usami M, Karino T, Komatsu S, Akasaki M. (2006) Skin tests of a novel nicotine patch, PHK-301p, in healthy male volunteers: phase I, placebo-controlled study. *J Pharmacol Sci.* **102**,72-6.
34. Palmer RA, White IR. (2006) Photopatch testing. In Frosch PJ, Menne T, Lepoittevin JP. (Eds.) *Contact Dermatitis. 4th ed.* Berlin:Springer. pp. 433-40.
35. Mano Y, Ishii T, Hotta K, Kusano K. (2015) Validation of a UPLC-MS/MS method for the simultaneous determination of E6005, a phosphodiesterase 4 inhibitor, and its metabolite in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci.* **998-999**:31-9.
36. Nakagawa H. (2006) Comparison of the efficacy and safety of 0.1% tacrolimus ointment with topical corticosteroids in adult patients with atopic dermatitis. *Clin Drug Invest.* **26**:235-46.
37. Houslay MD. (2010) Underpinning compartmentalized cAMP signaling through targeted cAMP breakdown. *Trends Biochem Sci.* **35**:91-100.

38. European Task Force on Atopic dermatitis. Severity scoring of atopic dermatitis: the SCORAD index (consensus report of the European Task Force on Atopic dermatitis). (1993) *Dermatology.* **186**:23-31.
39. Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M. (2001) The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. *Exp Dermatol.* **10**:11-18.
40. Saeki H, Furue M, Furukawa F, Hide M, Ohtsuki M, Katayama I, Sasaki R, Suto H, Takehara K, Committee for Guidelines for Management of Atopic Dermatitis of Japanese Dermatological Association. (2009) Guidelines for management of atopic dermatitis. *J Dermatol.* **36**:563-77.
41. Kawashima M, Harada S, Tango T. (2002) Evaluation of itch based on a new rating scale using patient diary. *Jpn J Clin Dermatol.* **56**:692−7. (in **Japanese**)
42. Kawashima M, Tango T, Noguchi T, Inagi M, Nakagawa H, Harada S. (2003) Addition of fexofenadine to a topical corticosteroid reduces the pruritus associated with atopic dermatitis in a 1-week randomized, multicentre, double-blind, placebo-controlled, parallel-group study. *Br J Dermatol.* **148**:1212–21.
43. Robichaud A, Stamatiou PB, Jin SLC, Lachance N, MacDonald D, Laliberté F, Liu S, Huang Z, Conti M, Chan CC. (2002) Deletion of phosphodiesterase 4D in mice shortens α2-adrenoceptor-mediated anesthesia, a behavioral correlate of emesis. *J Clin Invest.* **110**:1045-52.
44. Robichaud A, Tattersall FD, Choudhury I, Rodger IW. (1999) Emesis induced by inhibitors of type IV cyclic nucleotide phosphodiesterase (PDE IV) in the ferret. *Neuropharmacology.* **38**:289-97.
45. Schram ME, Spuls PI, Leeflang MMG, Lindeboom R, Bos JD, Schmitt J. (2012)

EASI, (objective) SCORAD and POEM for atopic eczema: responsiveness and minimal clinically important difference. *Allergy.* **67**:99-106.

46. O'Mahony L, Akdis M, Akdis CA. (2011) Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol.* **128**:1153-62.
47. Furue M, Kitahara Y, Akama H, Hojo S, Hayashi N, Nakagawa H; JAPANESE E6005 Study Investigators. (2014) Safety and efficacy of topical E6005, a phosphodiesterase 4 inhibitor, in Japanese adult patients with atopic dermatitis: results of a randomized, vehicle-controlled, multicenter clinical trial. *J Dermatol.* **41**:577-85.
48. Nemoto O, Hayashi N, Kitahara Y, Furue M, Hojo S, Nomoto M, Shima S; Japanese E6005 Study Investigators. (2015) Effect of topical phosphodiesterase 4 inhibitor E6005 on Japanese children with atopic dermatitis: Results from a randomized, vehicle-controlled exploratory trial. *J Dermatol.* **43**:881-7.